



FIG. 1a



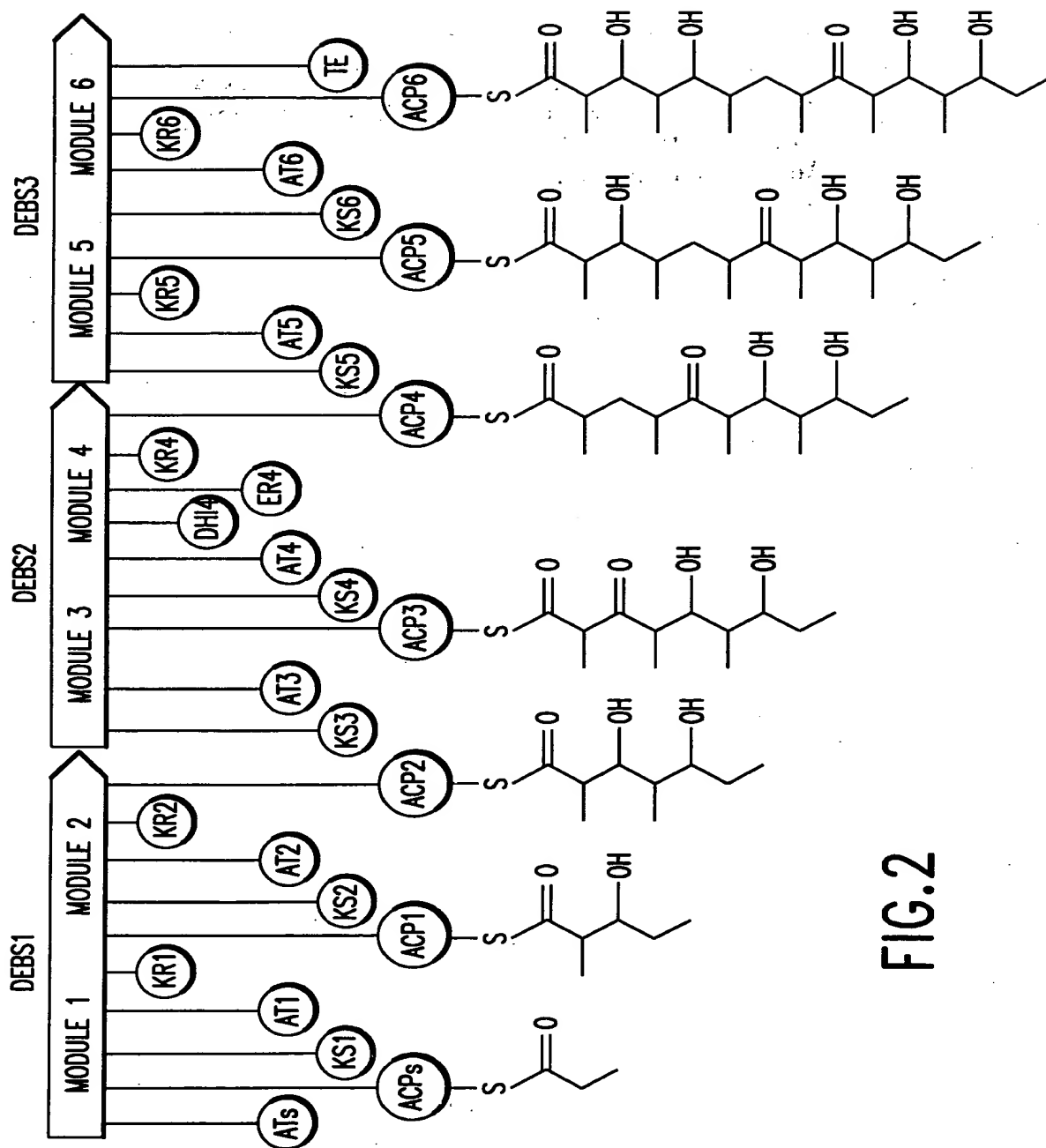


FIG.2

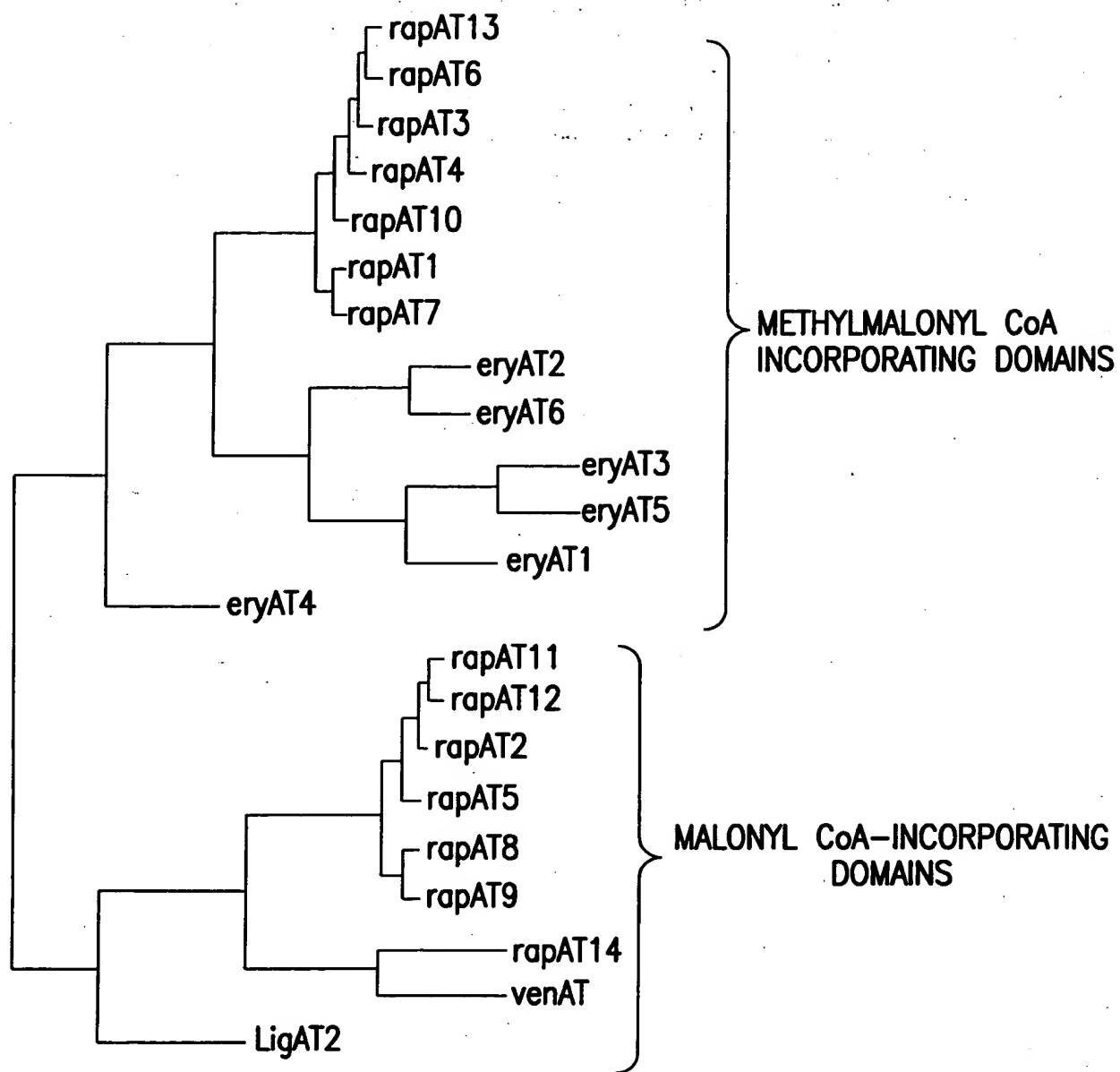
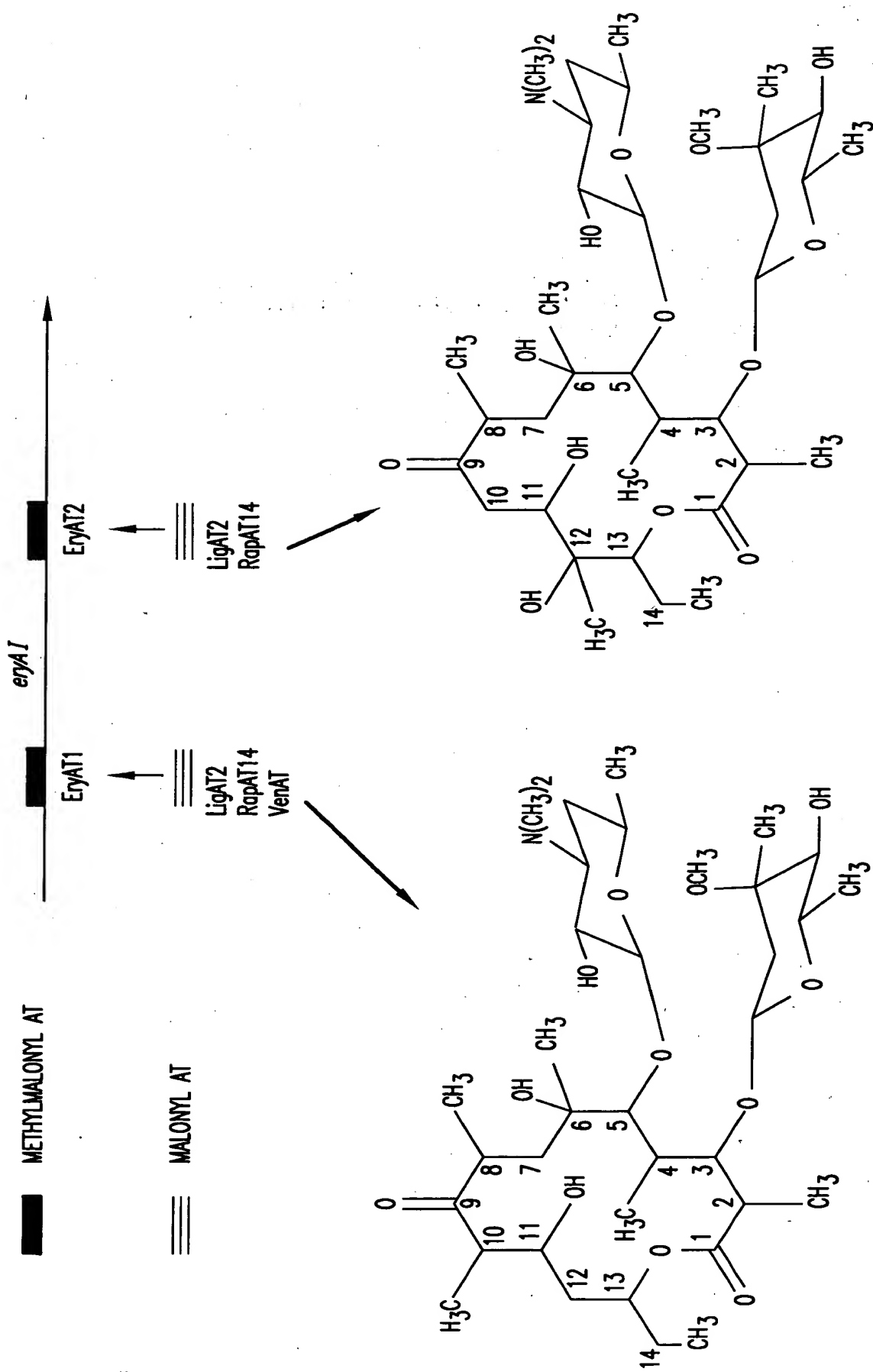


FIG.3



12-DESMETHYL, 12-DEOXYERYTHROMYCIN A

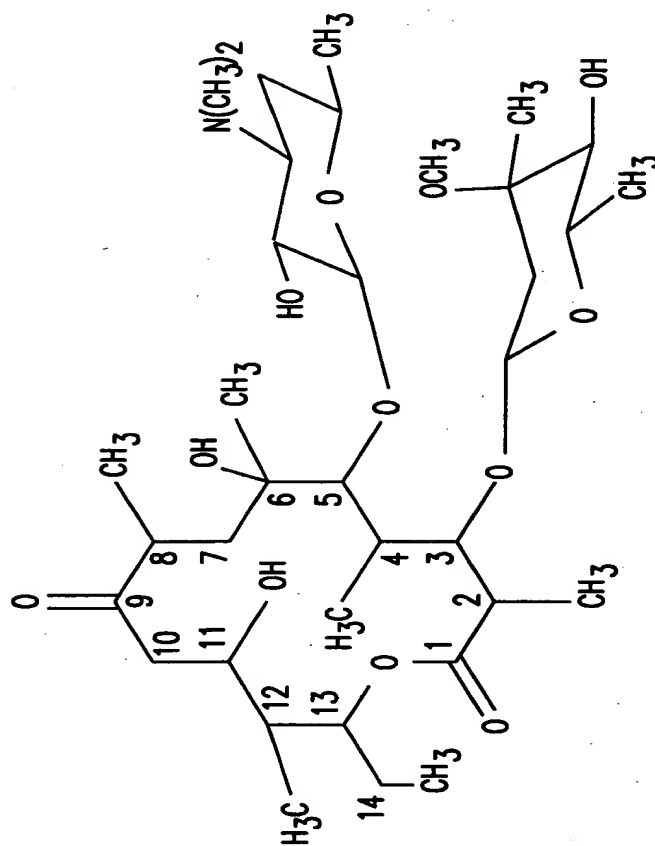
10-DESMETHYLERYTHROMYCIN A

AND

CONT. ON FIG.4b

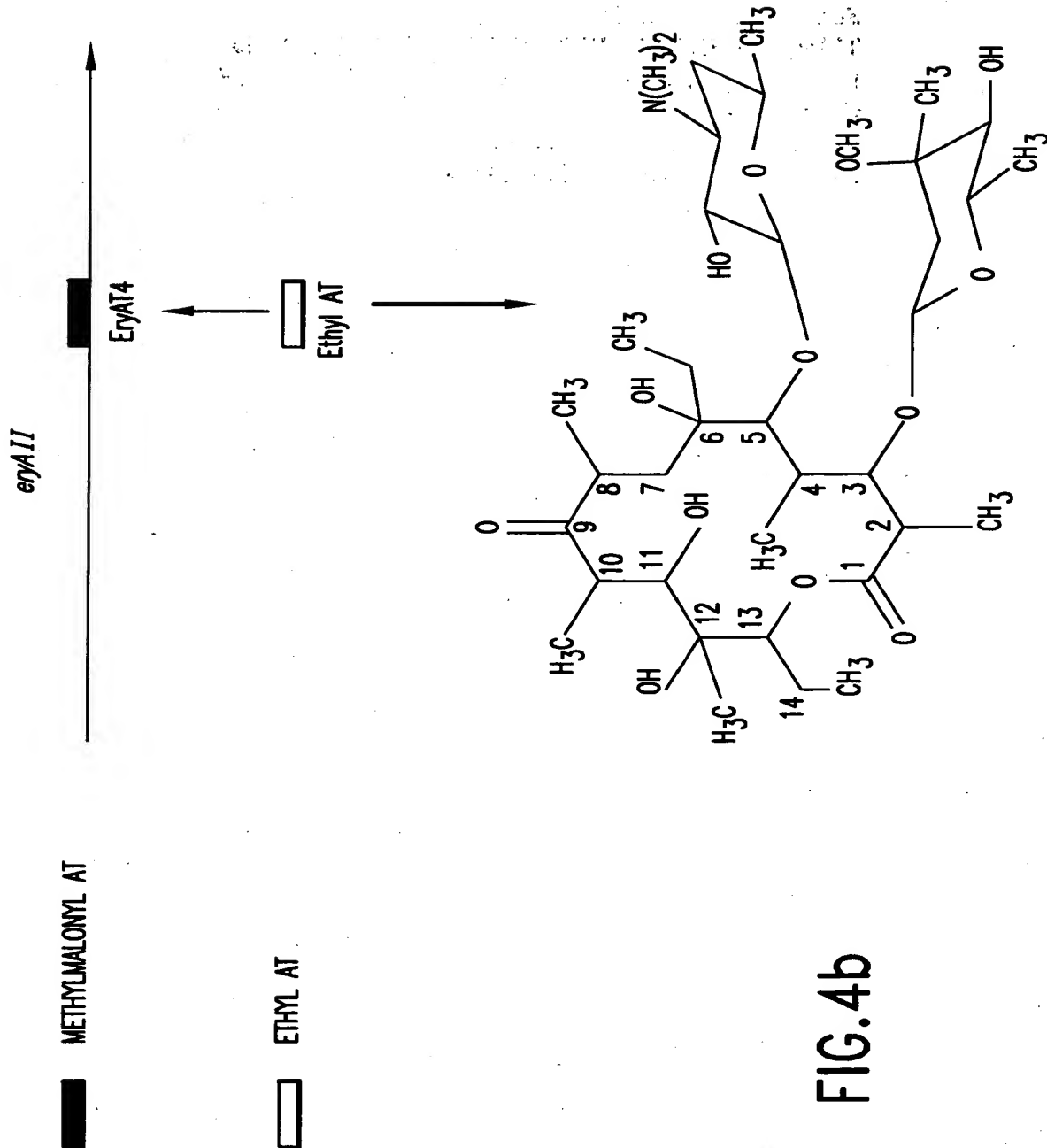
FIG. 4a(1)

CONT. FROM FIG. 4a



10-DESMETHYL-12-DEOXYERYTHROMYCIN A

FIG. 4a(2)



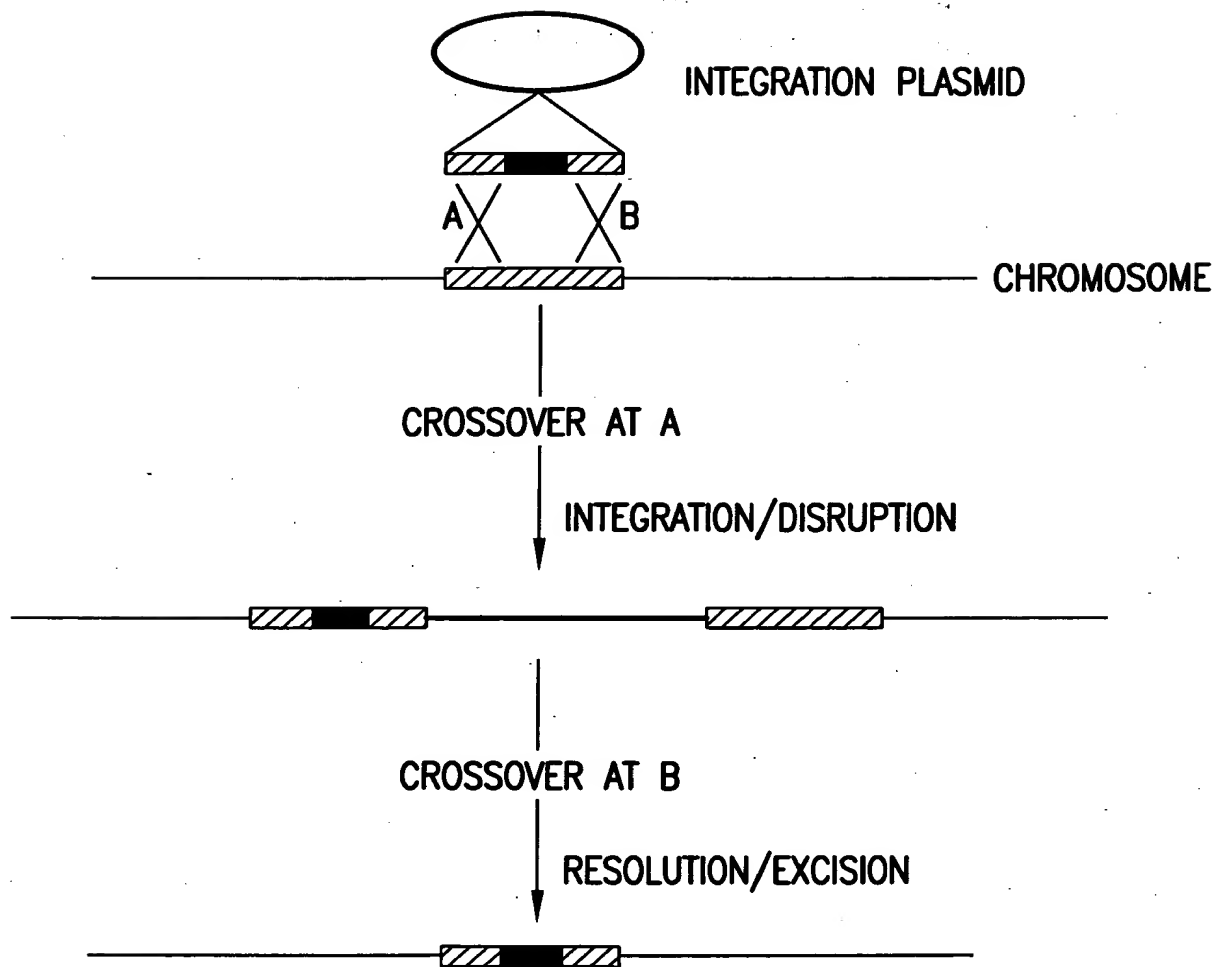


FIG.5

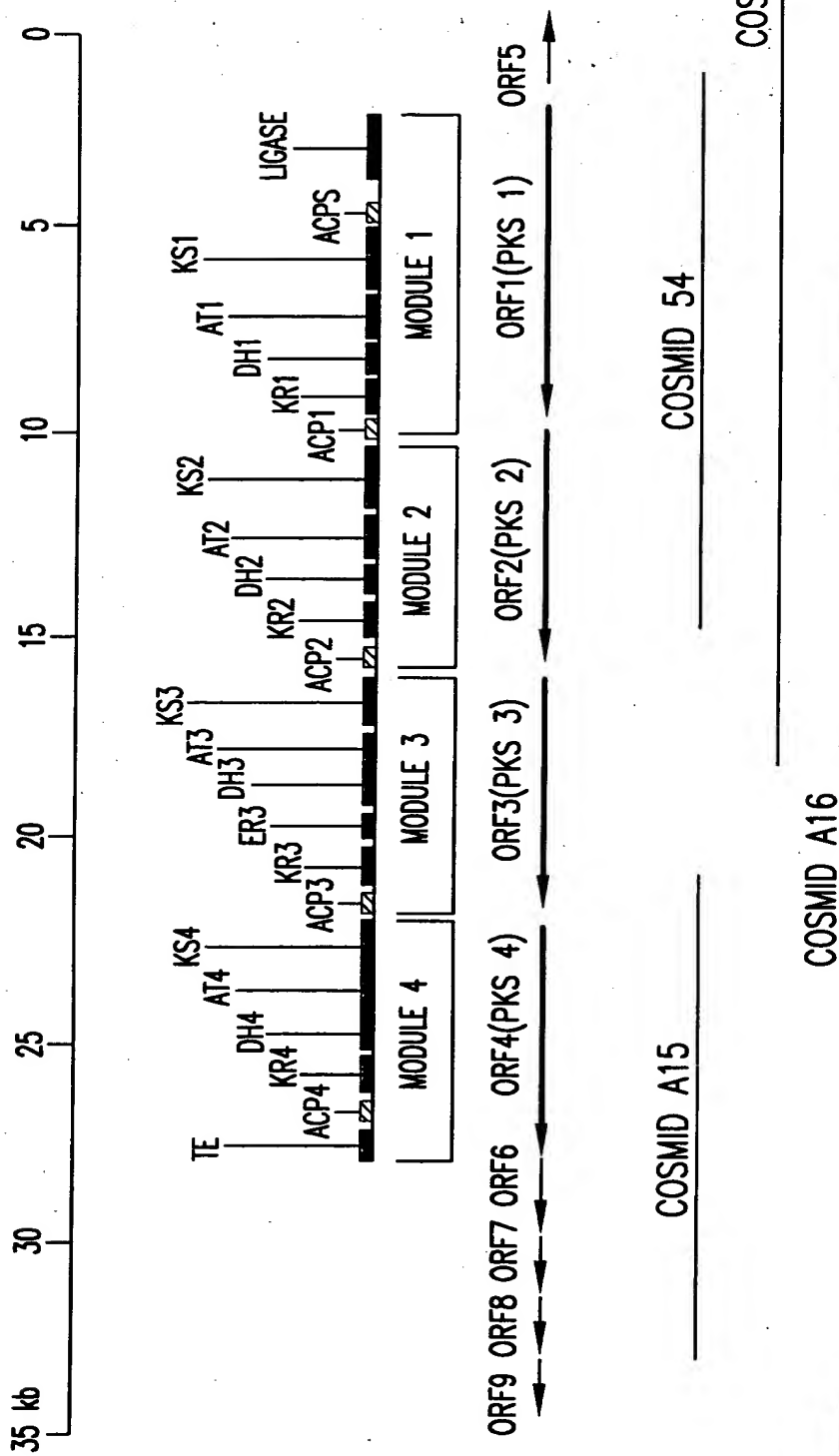
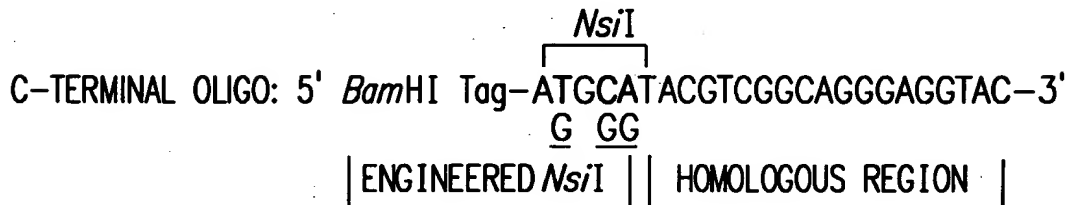
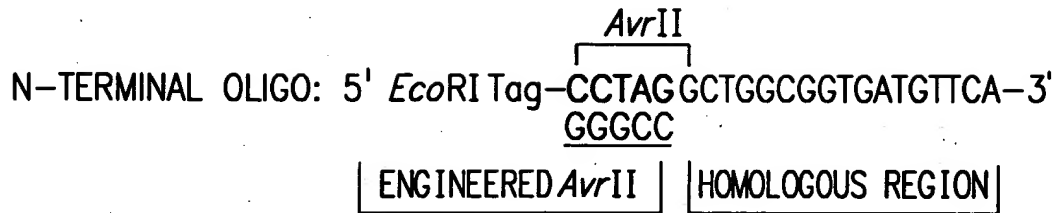


FIG.6

GGGCCGCTGGCGGTGATGTTACCGGACAGGGCTCCCAACGCCCCGGCATGGGACGACAG 60
 G P L A V M F T G Q G S Q R P G M G R Q 20
 TTGTACGAGCACTTCCCCGTCTSCGCCCAGGCACTGGACGAGGTCTTCGCACTCGCCACC 120
 L Y E H F P V F A Q A L D E V F A L A T 40
 CCCGACTACGCGAGGTGATGTTTCGACCCCGACCAGGGCGAAACACTCCAACGCACCGAC 180
 P G L R E V M F D P D Q A E T L Q R T D 60
 CACGCCCAGATCGCCCTGTTCCGCTTCGAAACCGCCCTCTACCGACTCTGGGAATCCTGG 240
 H A Q I A L F A F E T A L Y R L W E S W 80
 GGCCTGCGACCCGACATGGTCTGCGGACACTCGGTGCGAGAAATCACCGCAGCCACGTC 300
 G L R P D M V C G H S V G E I T A A H V 100
 TCCGGCACCCCTCACCTCCCCGACGCCGTCCACCTCGTCACCACACGCGGCACCCTCATG 360
 S G T L T L P D A V H L V T T R G T L M 120
 CAAAACCTGCCCCCGGGCGGCCATGCTCGCCGTGCCACCGACCCCCACACCCTCCAA 420
 Q N L P P G G A M L A V A T D P H T L Q 140
 CCCACCTCGACAACCACCACGACACCATCTCCATCGCCGCCATCAACGGCCCCCAGGCC 480
 P H L D N H H D T I S I A A I N G P H A 160
 ACCGTCTCTCCGGCGACCGCACCACCCTCCACCACATCGCCACCCAACCTCAACACCAAA 540
 T V L S G D R T T L H H I A T Q L N T K 180
 ACCAACTGGCTCAACGTCAGCCACGCCTTCCACTCCCCCTCATGCAACCCATCCTCCAA 600
 T N W L N V S H A F H S P L M Q P I L Q 200
 CCCTTCACCACCACCCTCAACACCCTACCCACCACCCCCCACACACCCCTCATCAGC 660
 P F T T T L N T L T H H P P H T P L I S 220
 ATGCTCACCGCCACACCCACCCACCCCGACACCACCCACTGGACCCAGCACATCACCGCA 720
 M L T A T P T H P D T T H W T Q H I T A 240
 CCCGTCCGCTACACCGACACCCTCCACCACCTCCACCACCACGGCATCACCACTACCTC 780
 P V R Y T D T L H H L H H H G I T T Y L 260
 GAAATCGGCCCCGACACCACCCTCACCGCCCTCGCCCGCACCACCCTCCCCACCACCACC 840
 E I G P D T T L T A L A R T T L P T T T 280
 CACCTCATCCCCACCACCCGCCGAACCACAACGAAGTCCGCAGCACGAACGAGGCGTTG 900
 H L I P T T R R N H N E V R S T N E A L 300
 GGCAGGGTGTTCAGCGTGGGCCACTCGGTGGACTGGCGGGCCCTCACTCCGACCGGGAGG 960
 G R V F S V G H S V D W R A L T P T G R 320
 CGTACCTCCCTGCCGACGTACCCCT 985
 R T S L P T Y P 328

FIG.7

PCR OLIGOS:



PCR CLONING:

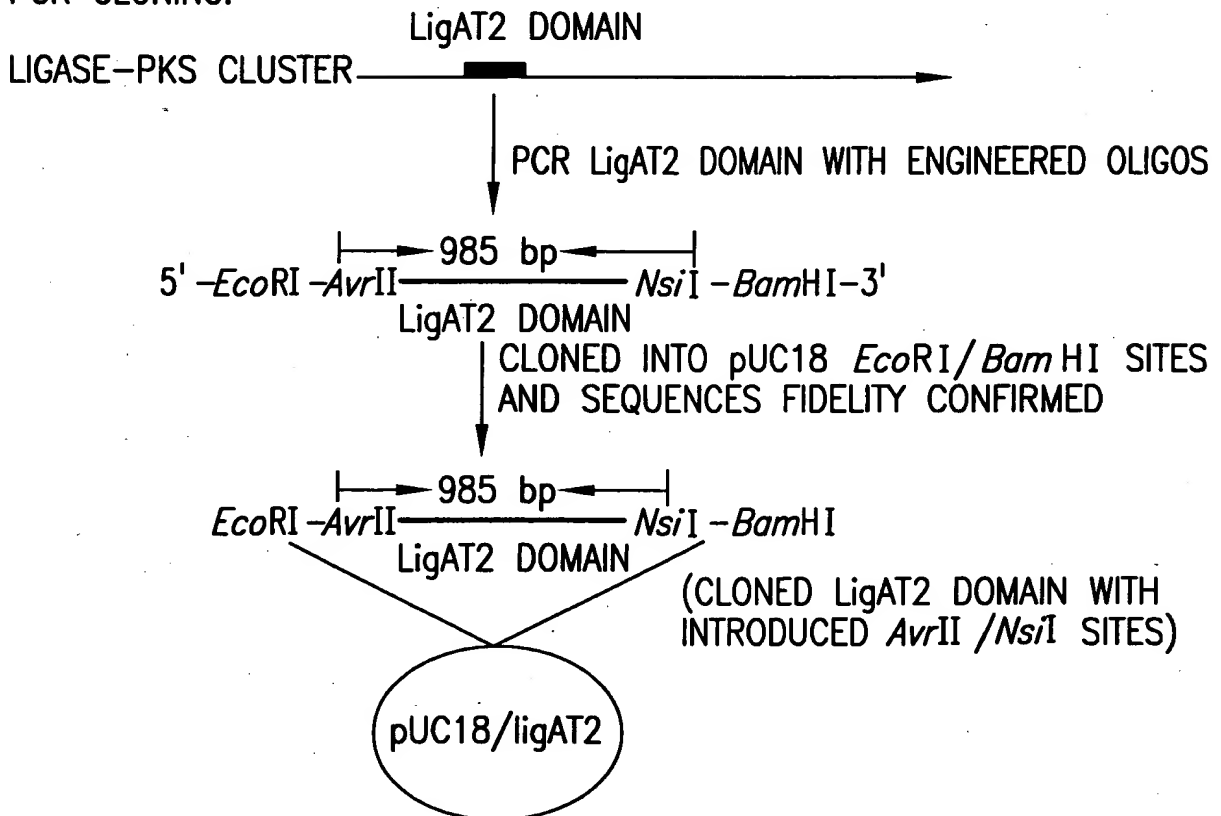


FIG.8

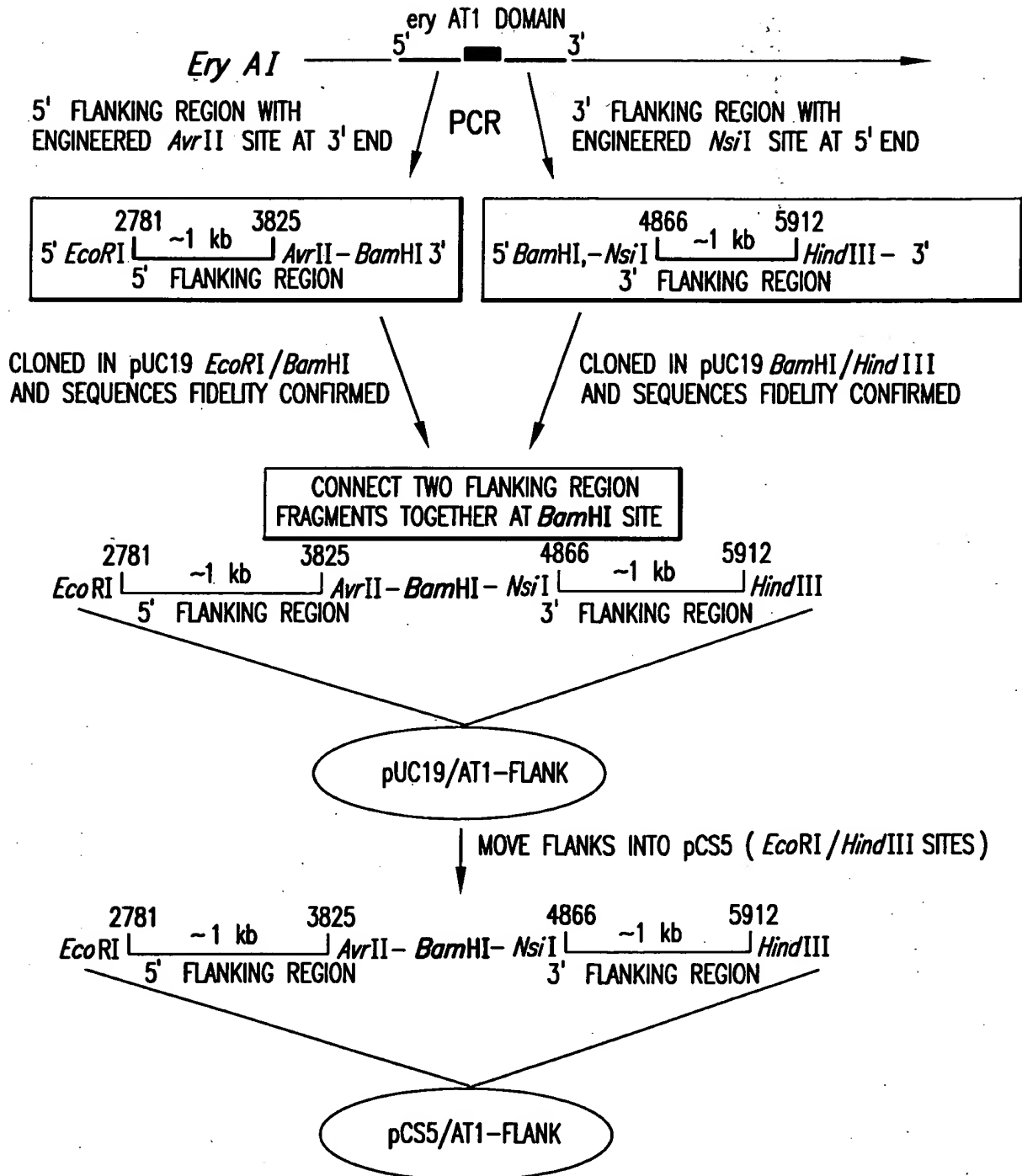


FIG.9

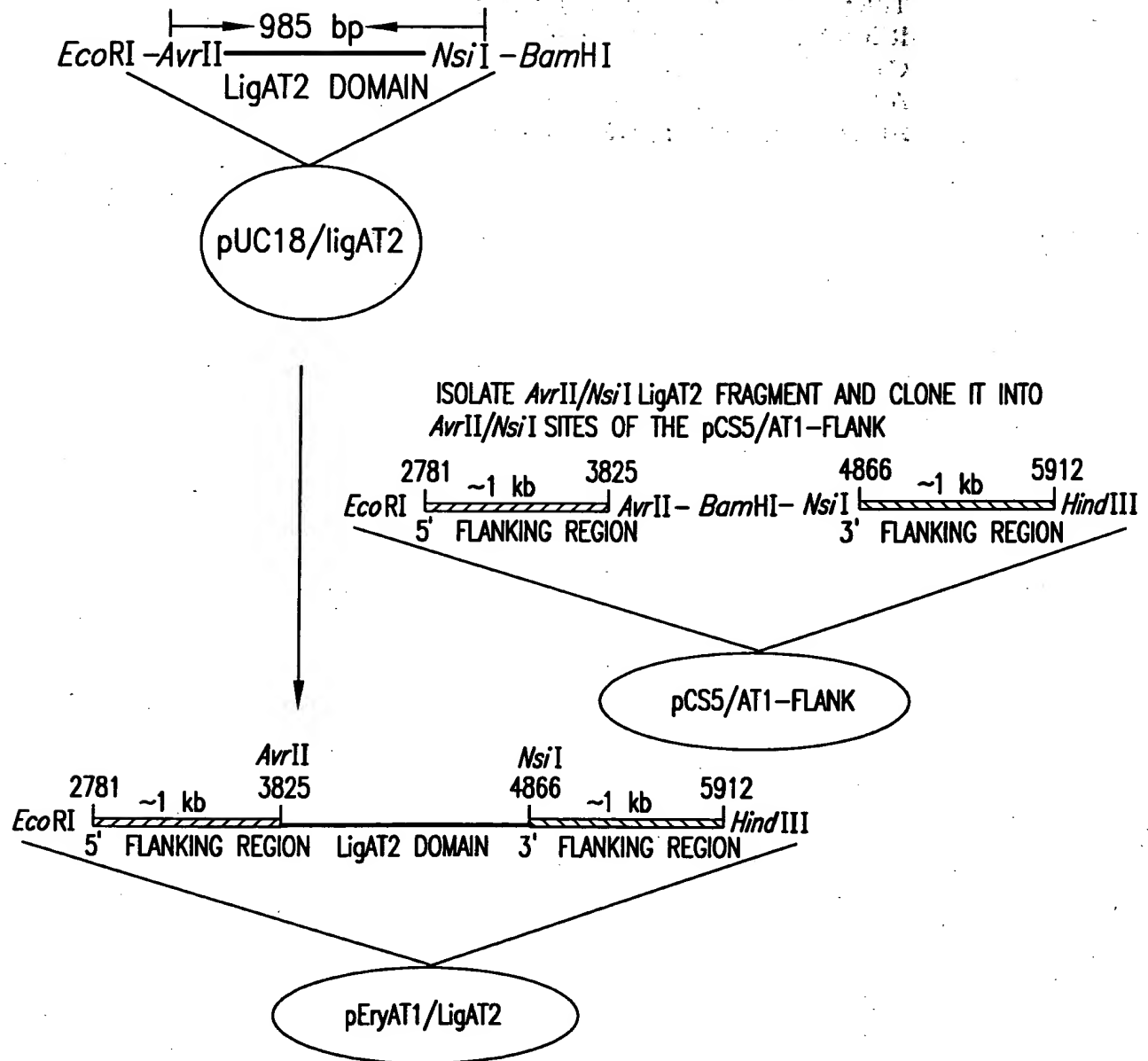


FIG.10

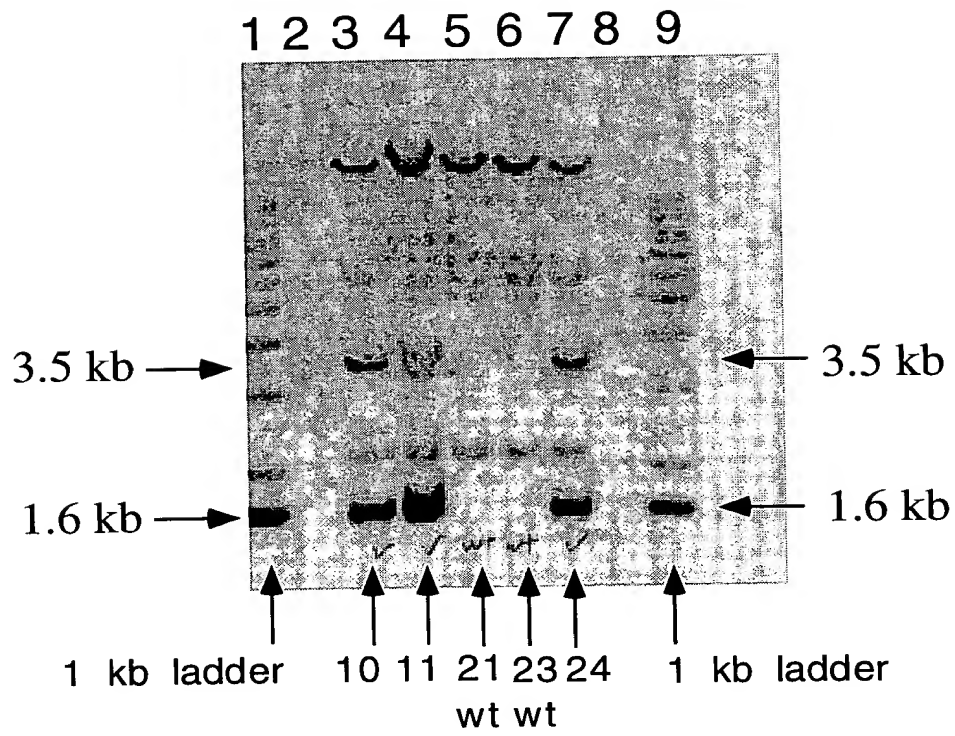


FIG. 11



1 2 3 4 5 6 7

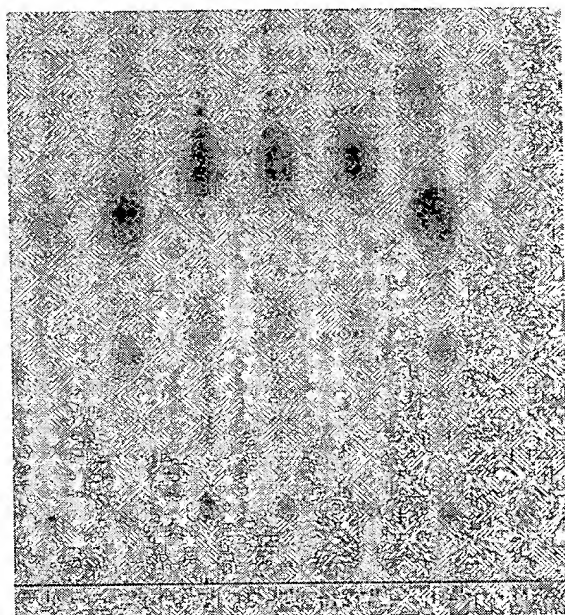


FIG. 12

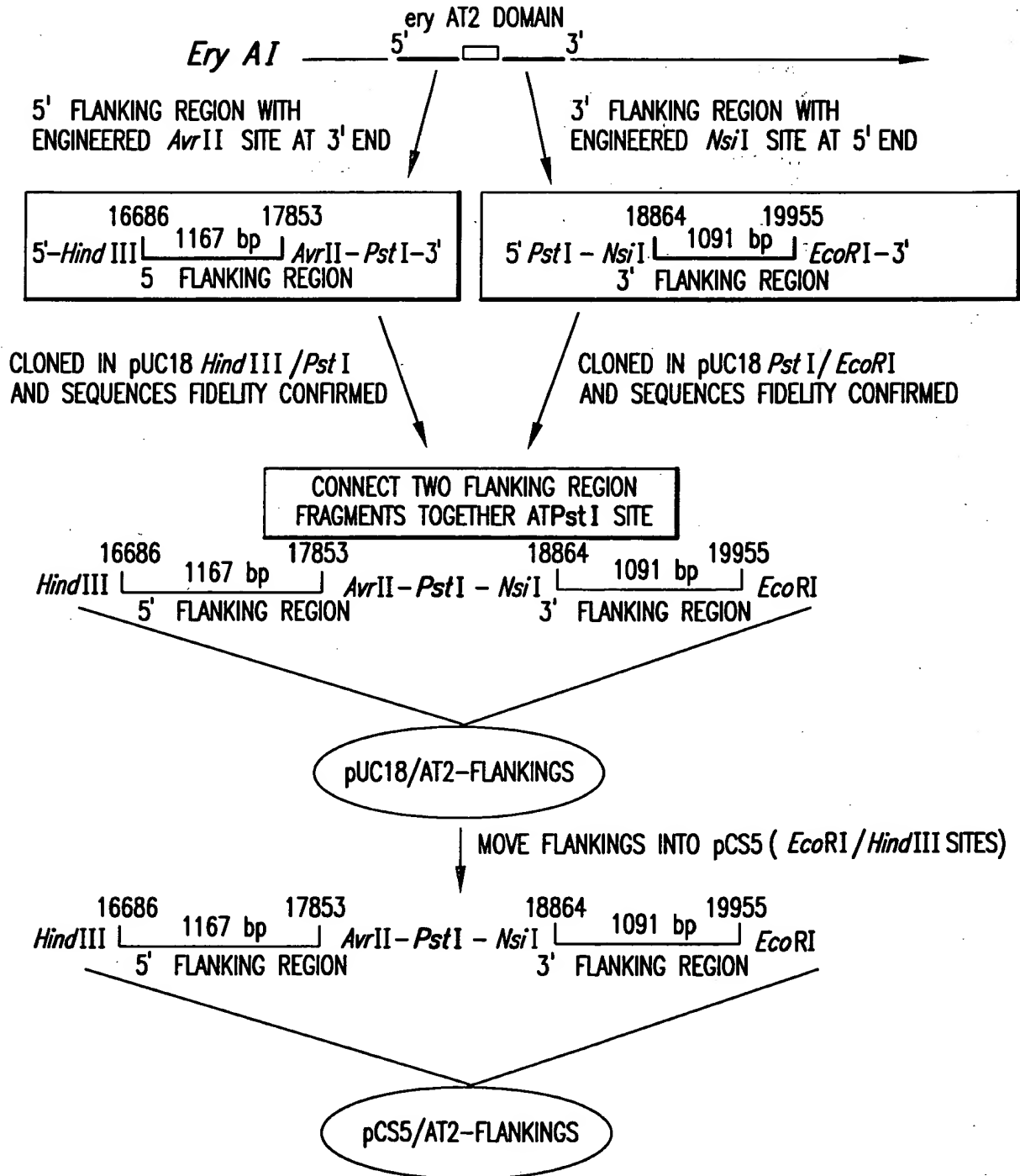
CONSTRUCTION OF *ery* AT2 FLANKING REGIONS IN pCS5

FIG.13

SCHEME FOR CONSTRUCTION OF pEryAT2/LigAT2 INTEGRATION PLASMID

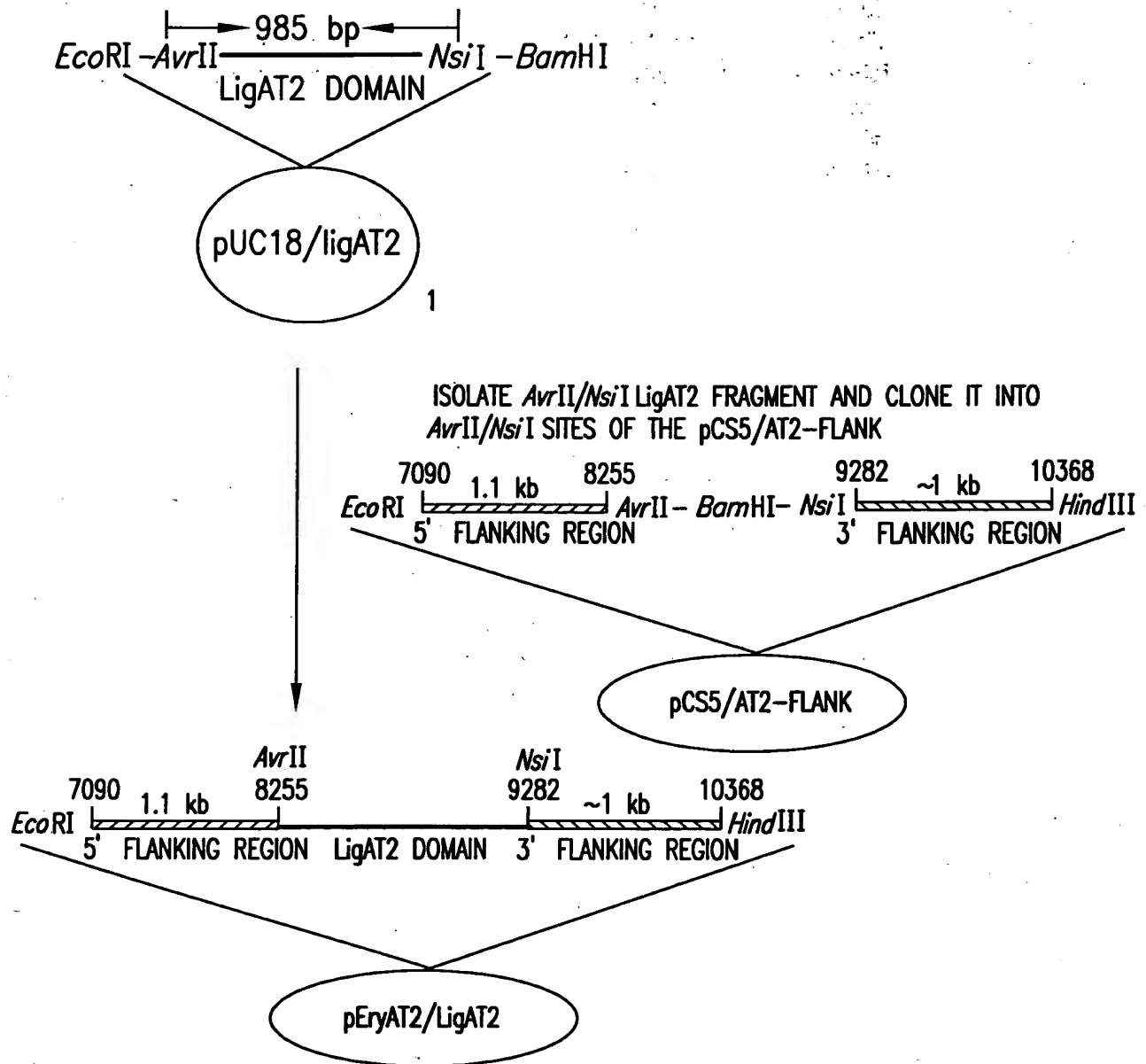


FIG.14

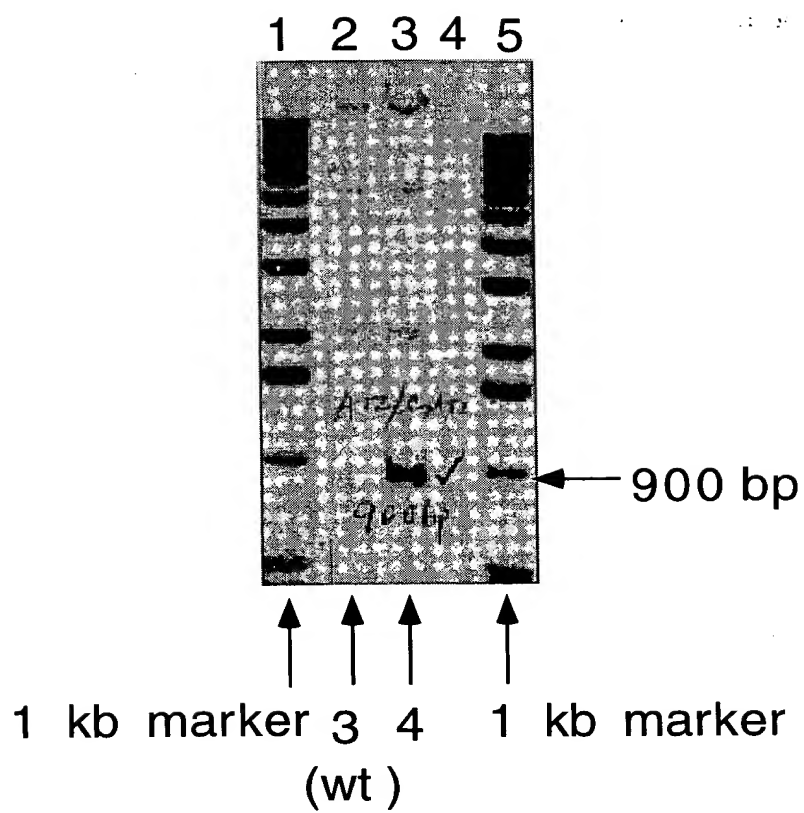


FIG. 15



1 2 3 4 5 6

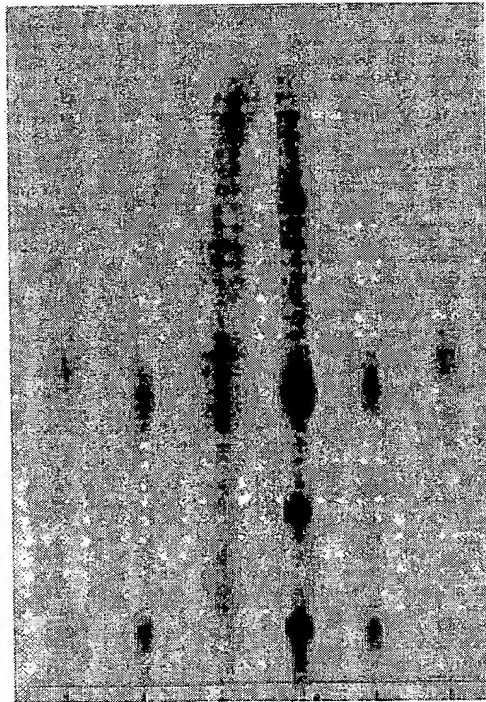


FIG.16

07-955-0505-60



95056268

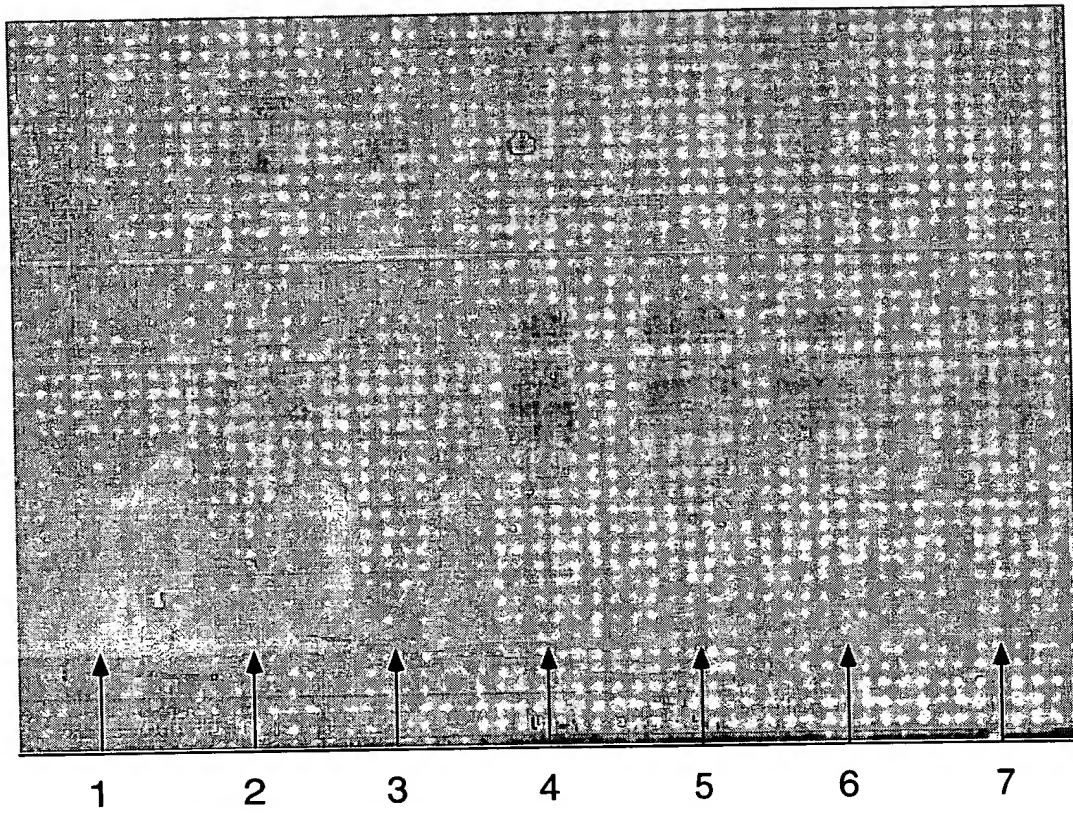


FIG. 17

CCTAGGACGGCAGTCCTGCTCACCAGGGCAGGGTTCCCAGCGTCAGGGCATGGGGCGCGAA 60
 P R T A V L L T G Q G S Q R Q G M G R E 20
 CTGTACGACCGGTACCGGTGTTCCGGCCTCGTTCCGACGCGATCTCGCTCAACTCGAC 120
 L Y D R S P V F A A S F D A I C A Q L D 40
 GGGCAACTGCCTCGTCCCCTCAAGGACGTTCTCTTCCGCCCCGAGGGGTCCGAGGACGCC 180
 G Q L P R P L K D V L F A P E G S E D A 60
 GCGCTCASCAGCCGTACGGTGTTACACAGGCGGCTCTGTTCCGCGTGAGACCTCCCTG 240
 A L I D R T V F T Q A A L F A V E T S L 80
 TTCCGGCTGTTCCGAGGCCACGGCCTCGSCCCGACTACCTCASCGGCCACTCCATCGGC 300
 F R L F E A H G L V P D Y L I G H S I G 100
 GAAGTGACCGCGGCCCGCTGCCCGGGTCTCGATCTGGCGGACGCGTCCGTCTGGTC 360
 E V T A A H L A G V L D L A D A C V L V 120
 GCCCACC CGCGCCCGCTGATGCAGTCGGCCCGGGCCGGCGCGGATGGCCCGGTCCAG 420
 A H R G R L M Q S A R A G G A M A A V Q 140
 GCGAGCGAGGACGAGGTACGCGAGGCCCTCGCGACCTTCGACGATGCGGTTCCCGTG GCC 480
 A S E D E V R E A L A T F D D A V A V A 160
 GGAGTCAACGGCCCCGAACGCCACCGTCGTCTCCGGCGACGAGGACGCGGTCCGAGCGGCTG 540
 G V N G P N A T V V S G D E D A V E R L 180
 GTCCGCGCTGGCGCGAGCAGGGCAGGCGGACGAAGCGGCTGCCGGTCAGCCACGCCTTC 600
 V A R W R E Q G R R T K R L P V S H A F 200
 CACTCGCCGCACATGGACGGGATCGTCGACGAGTTCTCGTACCGCCGTCTCCGGGCTCACC 660
 H S P H M I G I V D E F V T A V S G L T 220
 TTCCGCTCCCCGACGLTCCCGGTGCTCTCCAACGTACCGGGACCCTCGCCACCGTCGAC 720
 F R S P T I P V V S N V T G T L A T V D 240
 CACCTGACCTCGCCCGGTACTGGGCACGCCACATCCGCGAGGCCGTGCGCTTCGCCGAC 780
 Q L T S P A Y W A R H I R E A V R F A D 260
 GGGGTCCGTACCTGGAGGGCGAGGGCGTCACCGAATGGCTGGAGCTCGGGCCCCGACGGC 840
 G V R Y L E G E G V T E W L E L G P D G 230
 GTTCTCGTCGCCCTGGTCCGAGGACTGCCCTGGCGAAGGAGGCGGATCGCTCGCGTCCGCC 900
 V L V A L V E D C L A K E A G S L A S A 300
 CTGCGCAAGGGGGCGAGCGAGCCCCACACCGTGGGCGCGGCCATGGCCCGCGCGGTGCTG 960
 L R K G A S E P H T V G A A M A R A V L 320
 CGCGATCCGGCCCCGACTGGGCGGCGGTGTTCCCGGGCGCACGCGGGTCCGACCTCCG 1020
 R G S G P D W A A V F P G A R R V D L P 340
 ACGTATGCAT 1030
 T Y A 343

FIG. 18

N-TERMINAL OLIGO: 5' *Eco*RI Tag-CCTA ^{AvrII}GGACGGCAGTCCTGCTCACC-3'
GGCC
 | ENGINEERED *AvrII* | | HOMOLOGOUS REGION |

C-TERMINAL OLIGO: 5' *Bam*HI Tag-ATGCATACGTCGGAAGGTCGACCCG-3'

|ENGINEERED *Nsi*I || HOMOLOGOUS REGION |

OR CLONING:

Ven-PKS CLUSTER

venAT DOMAIN

PCR venAT DOMAIN WITH ENGINEERED OLIGOS

5' -EcoRI Tag- AvrII 1030 bp NsiI -BamHI Tag-3'

venAT DOMAIN

CLONED INTO pUC18 HincII SITES AND SEQUENCES FIDELITY CONFIRMED

[HincII] EcoRI -AvrII 1030 bp NsiI -BamHI [HincII]

venAT DOMAIN

(CLONED venAT DOMAIN WITH INTRODUCED AvrII /NsiI tag)

pUC18/venAT

FIG. 19

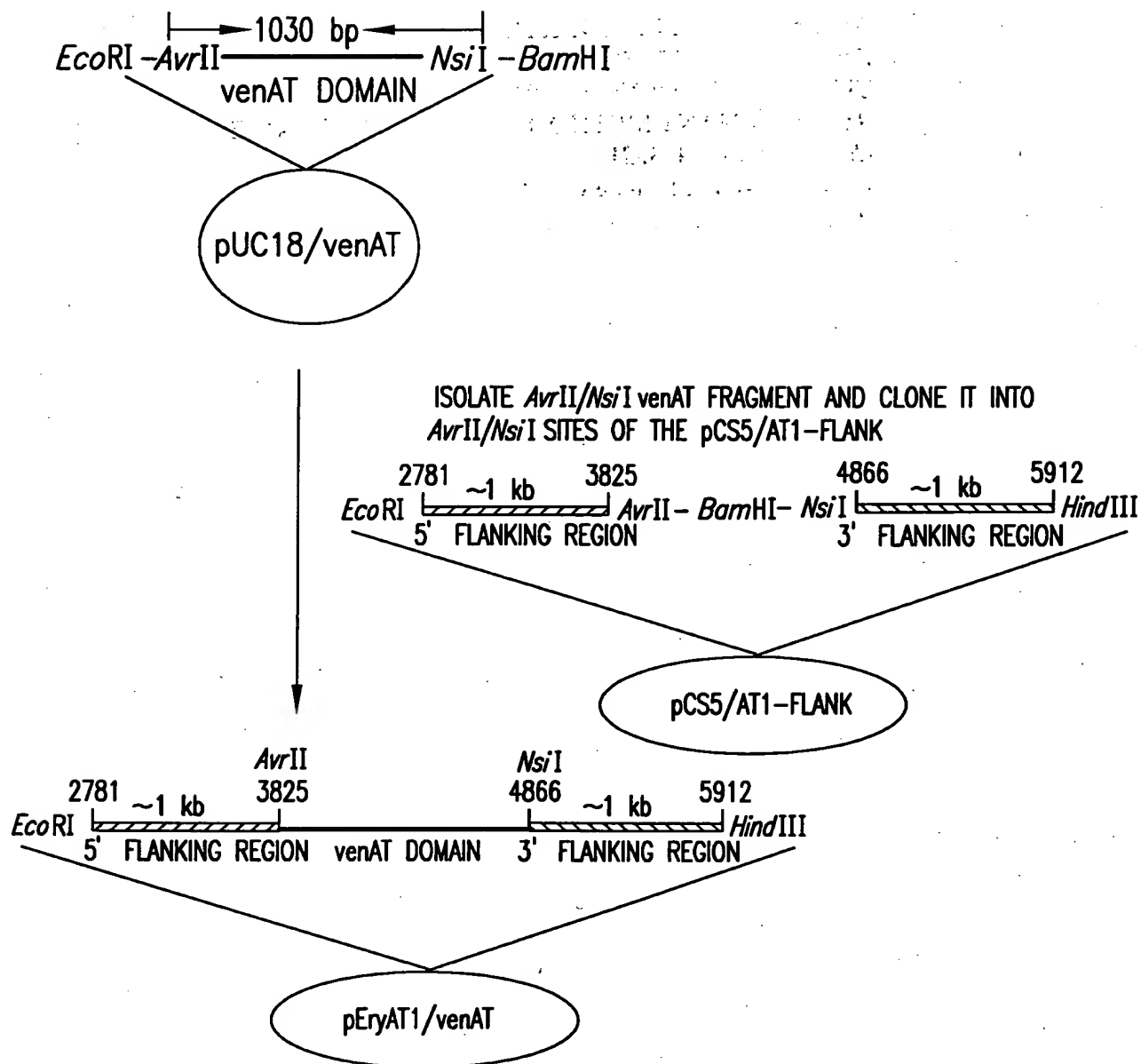


FIG.20

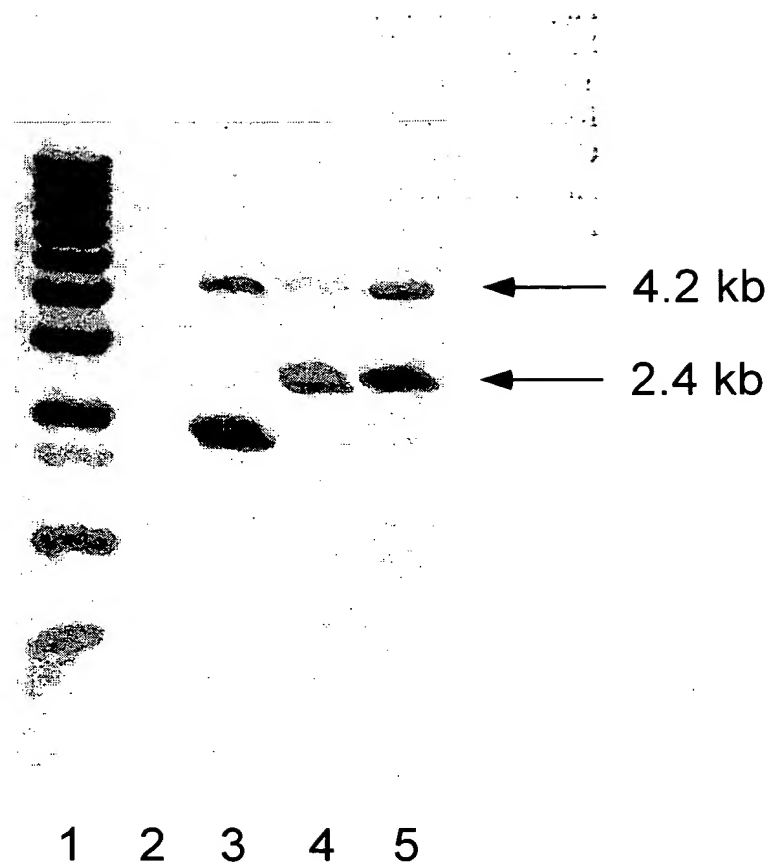


FIG. 21

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MEB

EryA



1 2

FIG. 22

PCR OLIGOS:

N-TERMINAL OLIGO: 5' *Eco*RI Tag-^{*Avr*II}
^{GGC C}
 CCTAGGGTTGCCTTCCTGTTTCGAC-3' (SEQ. ID NO. 17)

ENGINEERED *Avr*II | HOMOLOGOUS REGION

C-TERMINAL OLIGO: 5' *Hind*III Tag-^{*Nsi*I}
^{C G}
 ATGCATAGACCGGCAGATCCACCG-3' (SEQ. ID NO. 18)

ENGINEERED *Nsi*I | HOMOLOGOUS REGION

PCR CLONING:

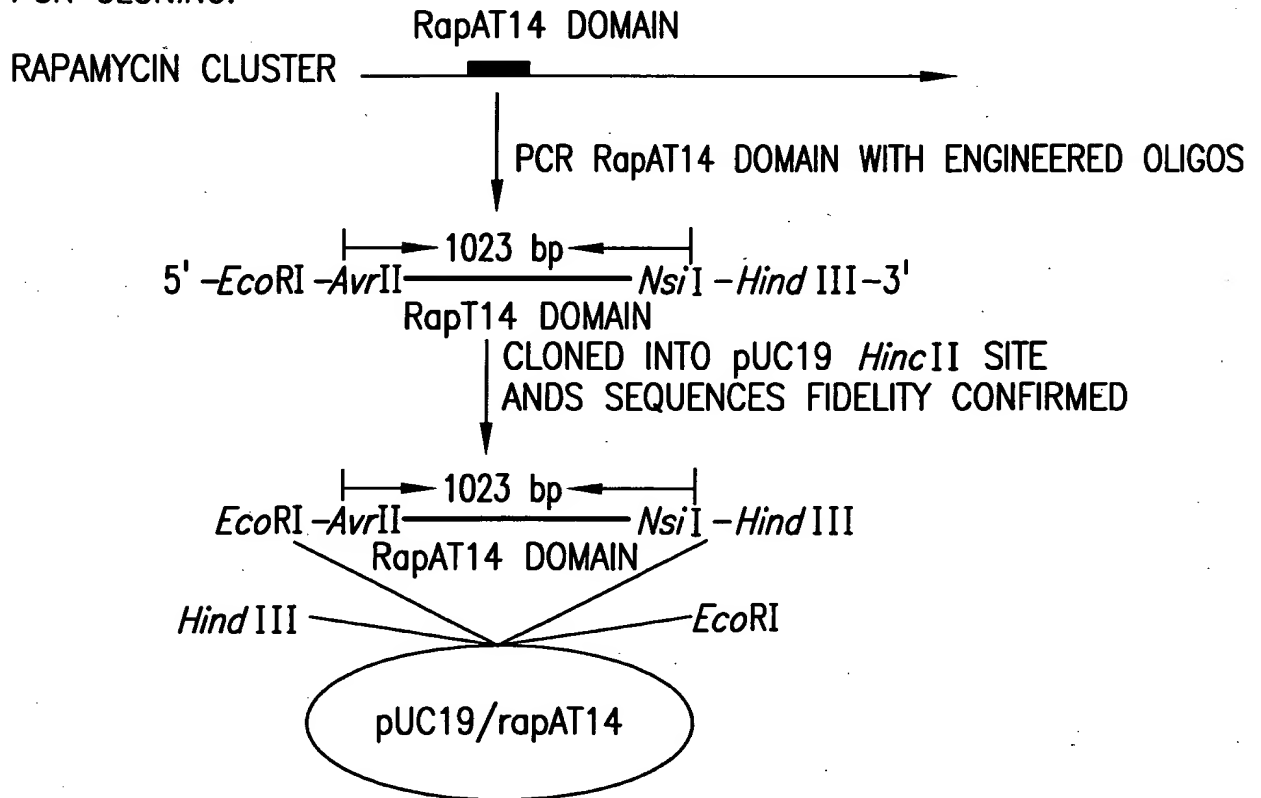


FIG.23

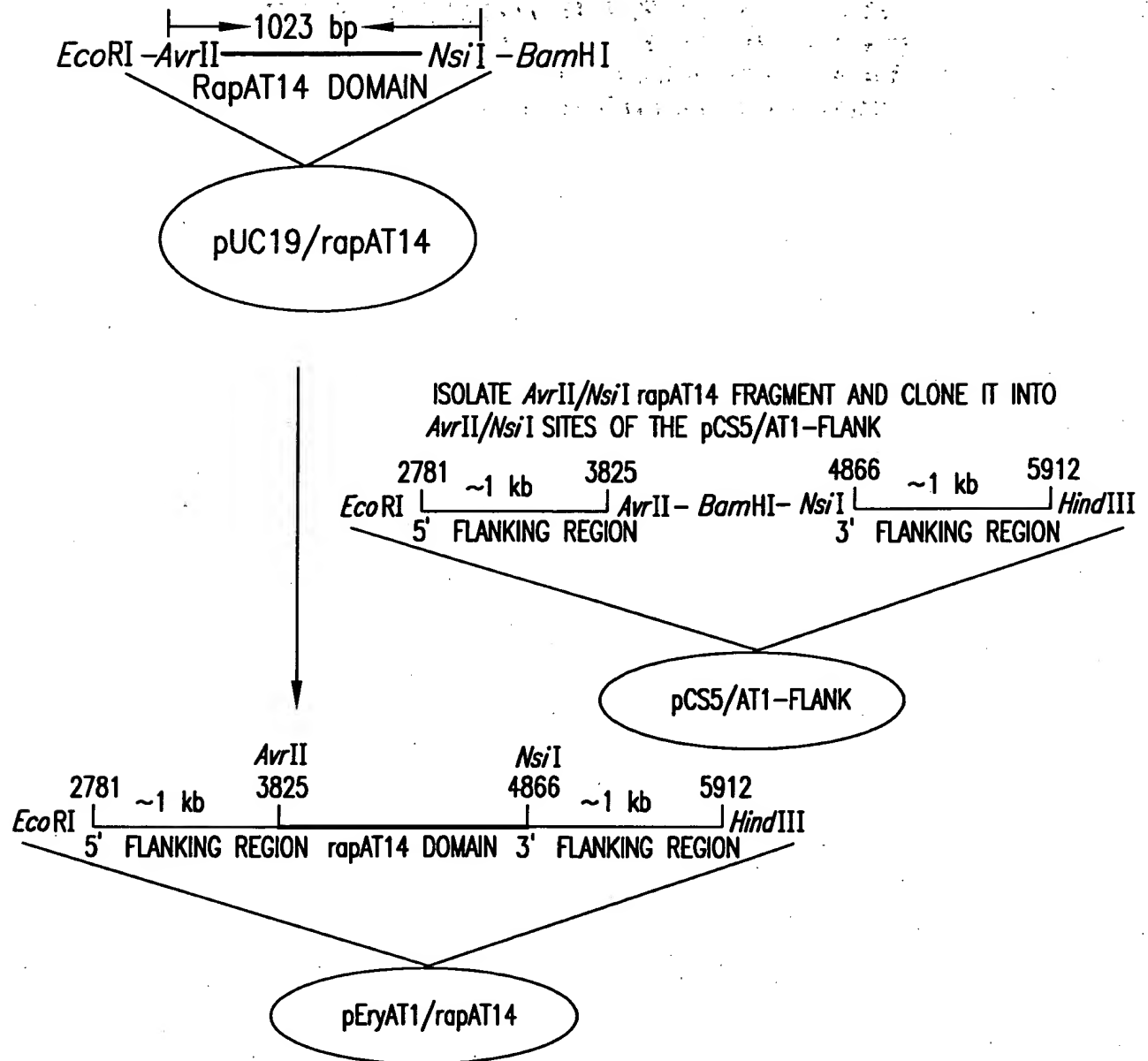


FIG.24

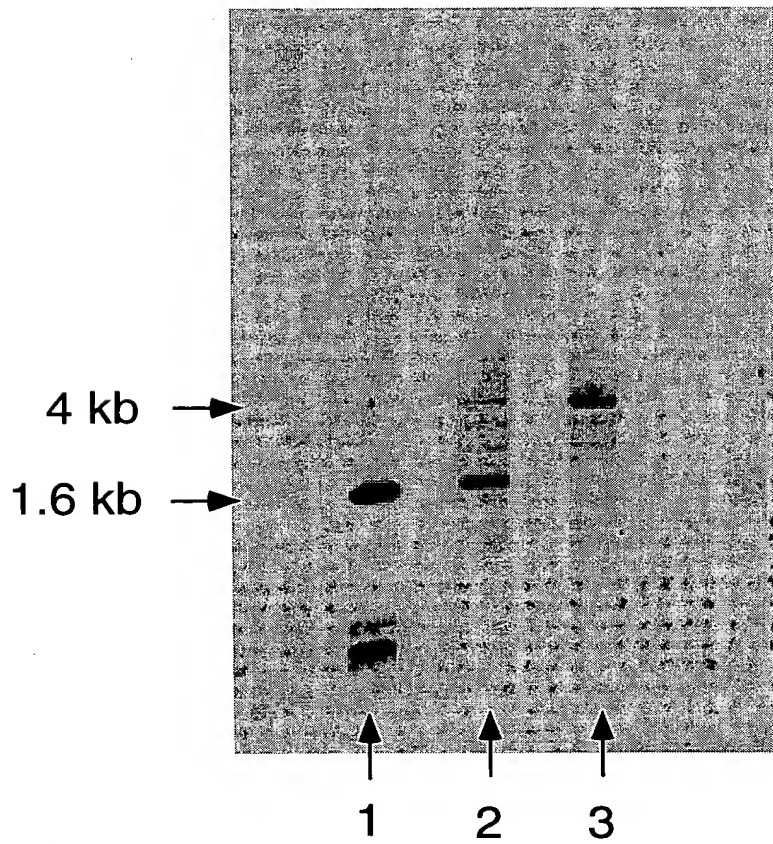


FIG. 25



2

FIG. 26

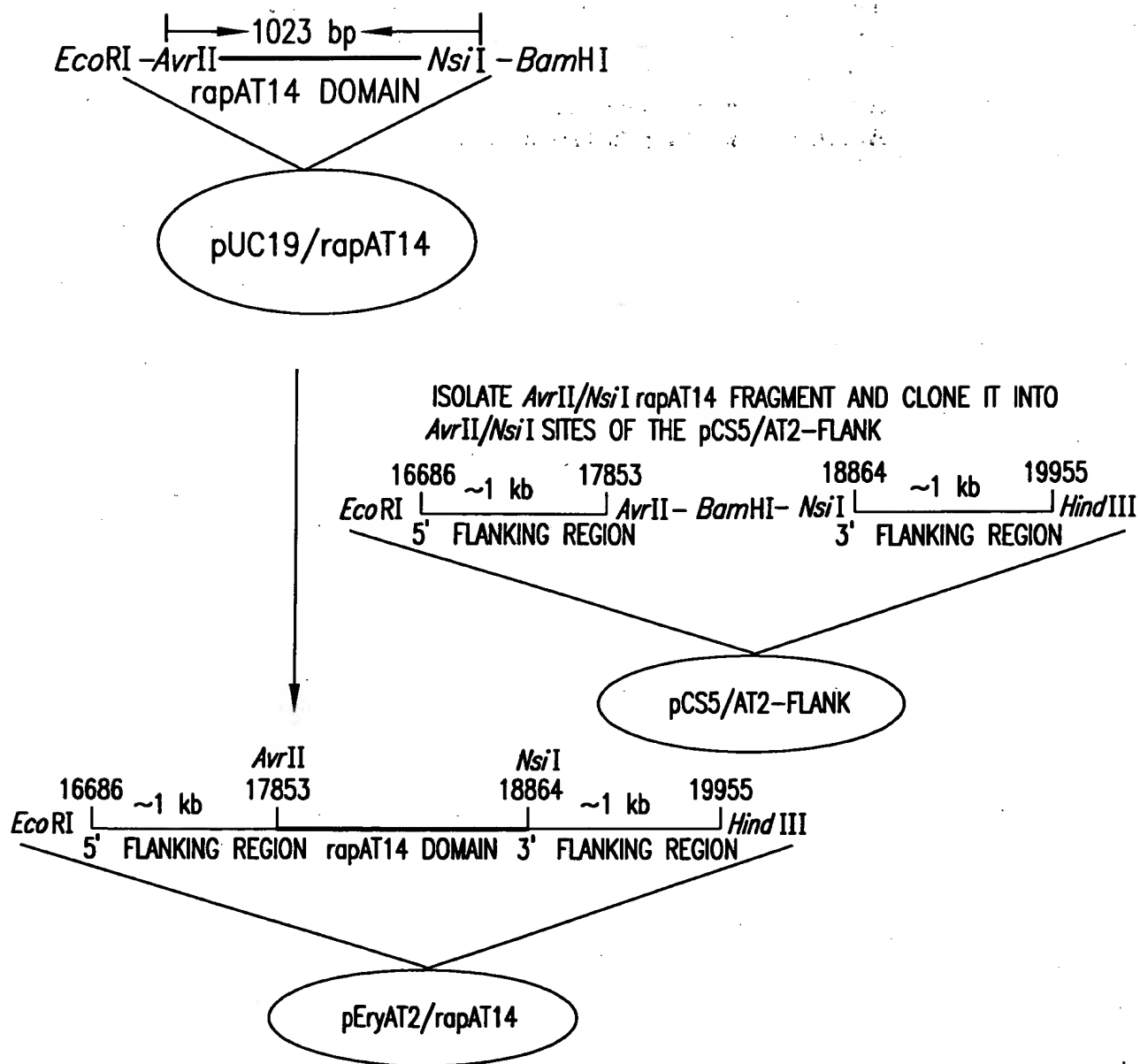


FIG.27

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← 4.3 kb

← 1.1 kb

1 2 3 4 5 6 7

FIG. 28

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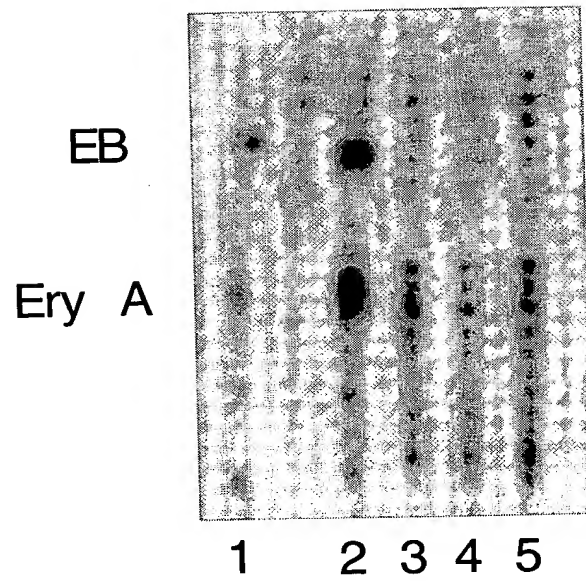


FIG. 29

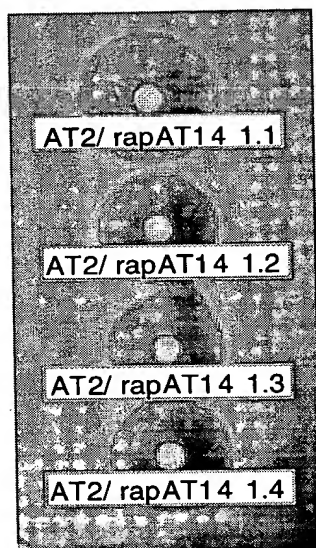
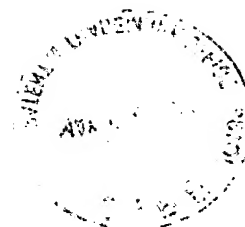


FIG. 30

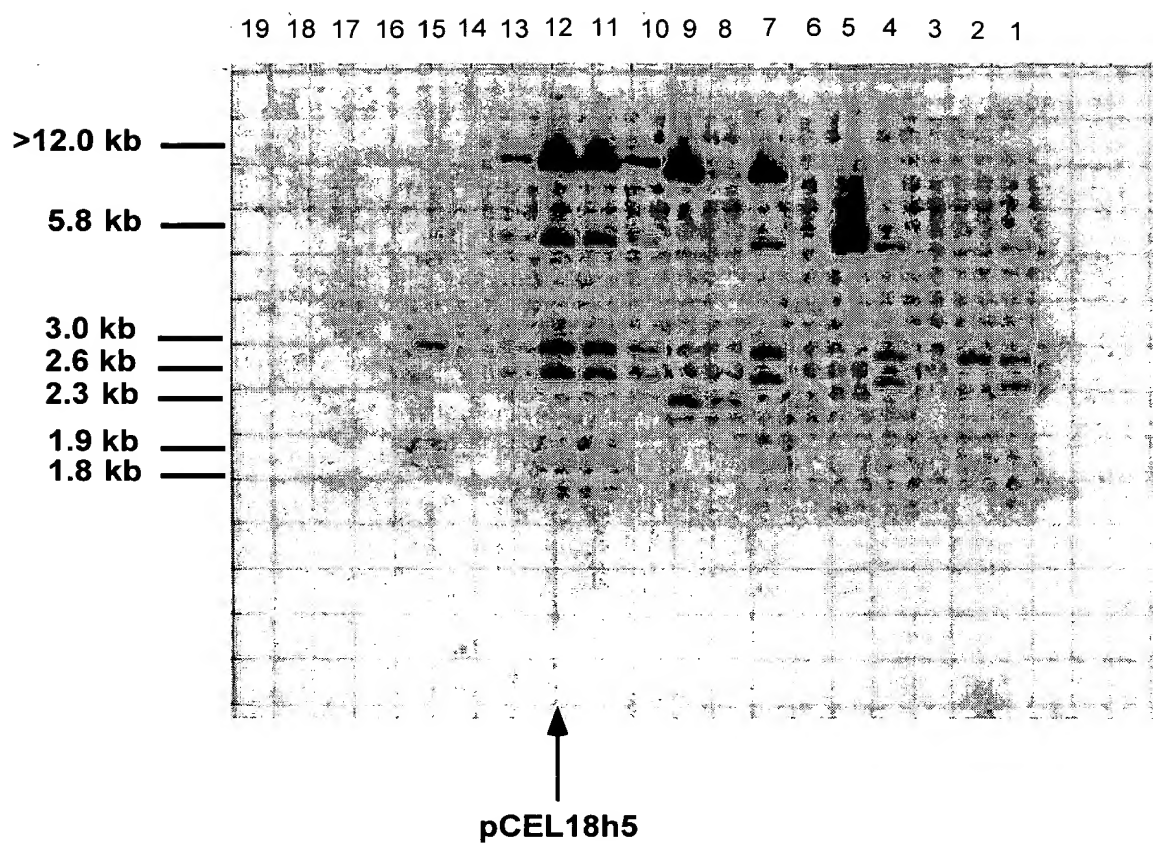
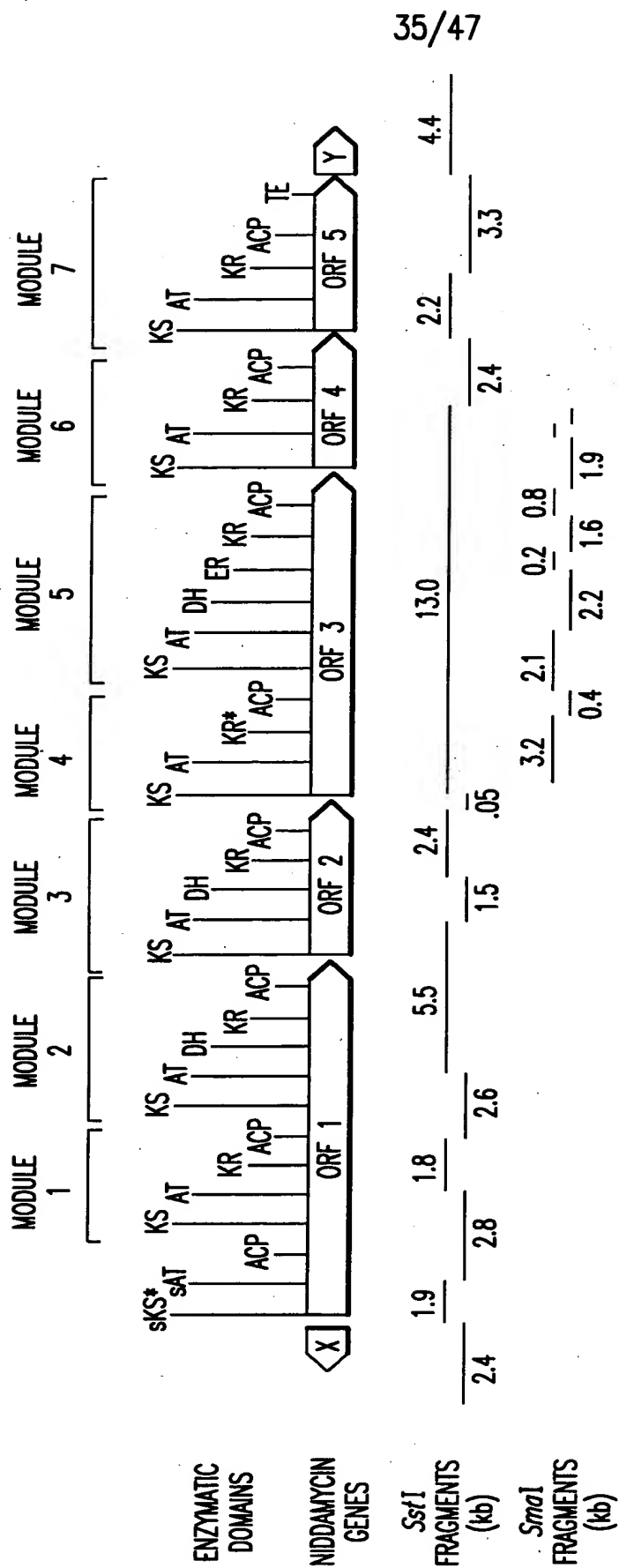


FIG. 31



pCEL18h5

pCEL13f5

FIG.32

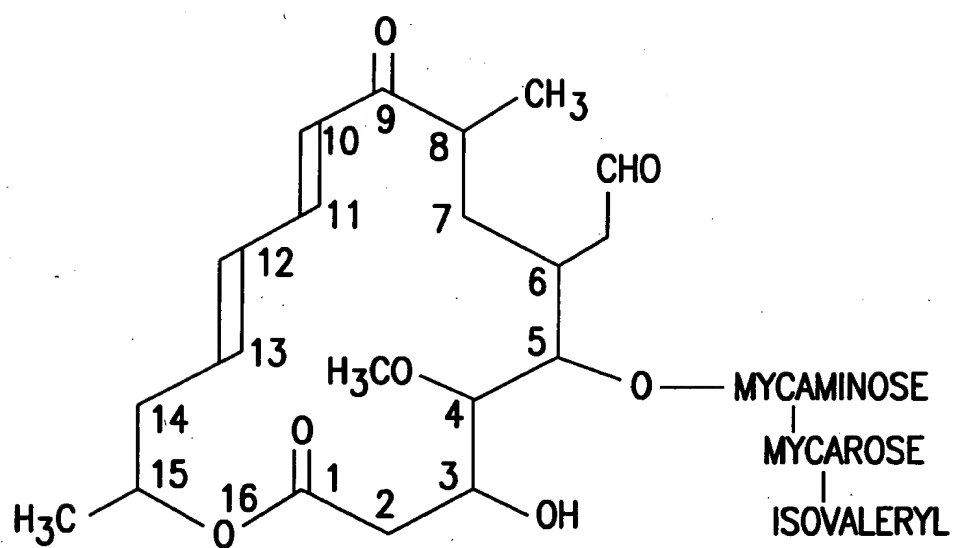


FIG.33

GCCGACCGTGTCGTGTTTCGTGTTCCCCGGCCAGGGCTCGCAGTGGGCCGGAATGGCCGAG 60
 A D R V V F V F P G Q G S Q W A G M A E 20
 GGGCTGCTGGAGCGGTCCGGCGCGTTCGGAGTGGGCCGACTCGTGCGACGCCGCGCTG 120
 G L L E R S G A F R S A A D S C D A A L 40
 CGGCCGTACCTCGGCTGCTCGGTGCTGAGCGTGTGCGCGGGGAACCGGACGCGCCCTCG 180
 R P Y L G W S V L S V L R G E P D A P S 60
 CTCGACCGGTCGACGTGCTGCAGCCGGTGTGTTACGATGATGGTCTCGCTCGCGGCG 240
 L D R V D V V Q P V L F T M M V S L A A 80
 GTCTGGCGTGGCTGGGCGTGGAACCGGCGGCGTGTGCGGCACTCGCAGGTGAGATC 300
 V W R A L G V E P A A V V G H S Q G E I 100
 GCCGCTGCCCATGTGCGCGGTGCGCTGTGCTGGACGACTCGGCCCCGATCGTGCCCTG 360
 A A A H V A G A L S L D D S A R I V A L 120
 CGCAGTCGGGCGTGGCTCGGACTGGCGGGCAAGGGCGGCATGGTGGCGGTGCCGATGCCG 420
 R S R A W L G L A G K G G M V A V P M P 140
 GCGGAGGAGCTGCGGCCGCGGCTGGTGACGTGGGGGACCGTCTGGCCGTGCGCGCCGTC 480
 A E E L R P R L V T W G D R L A V A A V 160
 AACAGCCCCGGTTCCTGCGCCGTGCGAGGCGACCCGGAGGCGCTGGCCGAACCTGGTGGCG 540
 N S P G S C A V A G D P E A L A E L V A 180
 CTGCTGACCGGTGAGGGGTGCACGCCCCGGCGATCCCCGGCGTGCACACGGCGGGCCAC 600
 L L T G E G V H A R P I P G V D T A G H 200
 TCGCCGCAGGTGGACGCGTTGCGGGCTCATCTGCTGGAGGTGCTGGCCCCGGTCCCCCCC 660
 S P Q V D A L R A H L L E V L A P V A P 220
 CGACCGGCCGACATCCCGTTCTACTCGACGGTGACCGGCGGGCTGCTGGACGCCACCGAG 720
 R P A D T P F Y S T V T G G L L D G T E 240
 CTGGACGCGACGTACTGGTACCGCAACATGCGCGAGCCCGTCGAGTTCGAGCGGCCACA 780
 L D A T Y W Y R N M R E P V E F E R A T 260
 CGGGCGCTGATCGCCGACGGGCACGACGTCTTCTGGAGACGAGCCCGCATCCCATGCTG 840
 R A L I A D G H D V F L E T S P H P M L 280
 GCCGTGGCGCTGGAGCAGACGGTCACCGACGCGCGCACCGACGCGGCGGTGCTCGGGACC 900
 A V A L E Q T V T D A G T D A A V L G T 300
 CTGCGCCGCGCCACGGCGGTCTCGCGCGTGGCCCTGGCCGTCTGCCGCGCCTTCGCG 960
 L R R R H G G P R A L A L A V C R A F A 320
 CACGGCGTGGAGGTGGACCCCGAGGCGGTCTTCGGTCCGGGCGCACGGCCCGTGGAGTTG 1020
 H G V E V D P E A V F G P G A R P V E L 340
 CCCACCTATCCG 1032
 P T Y P 344

FIG. 34

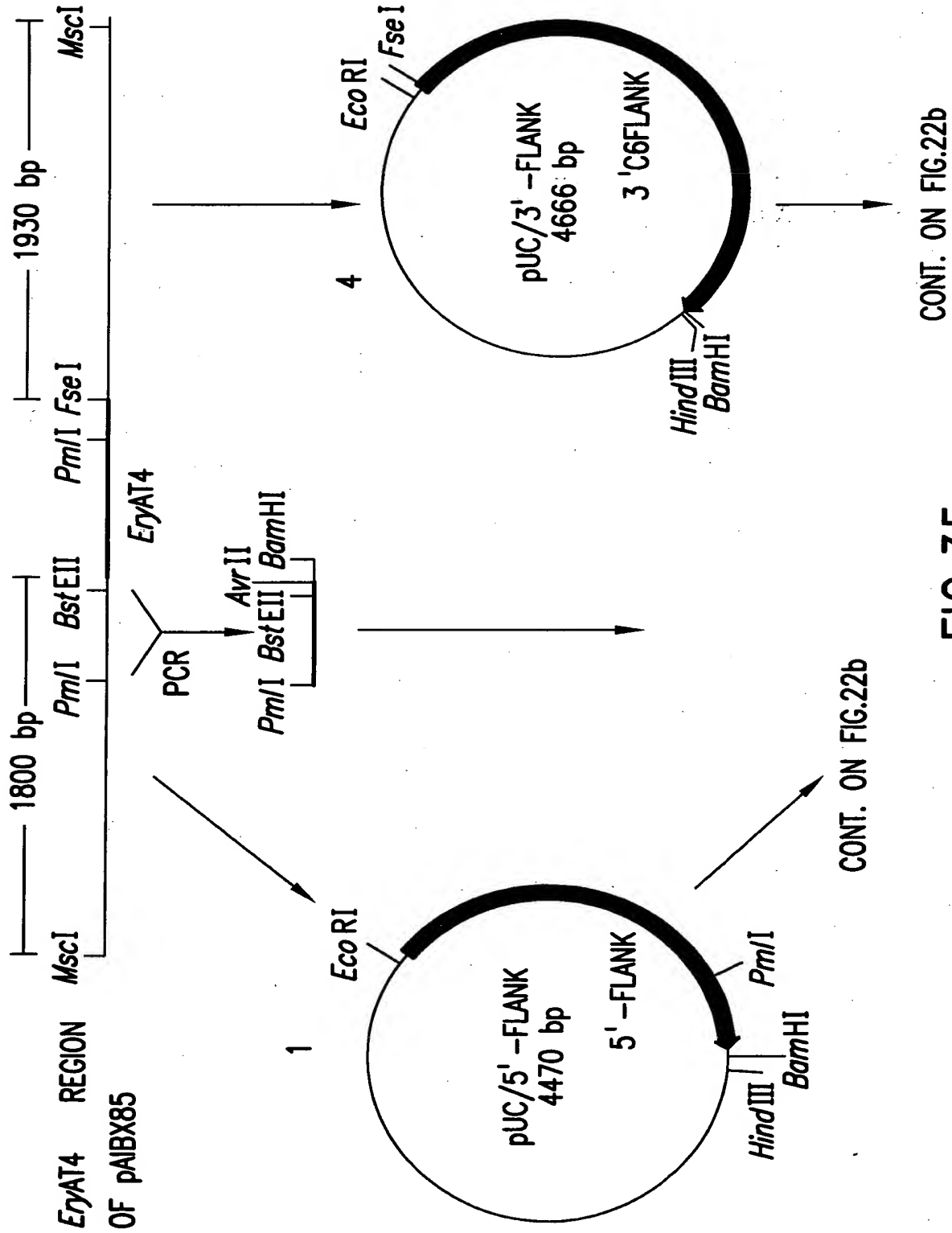
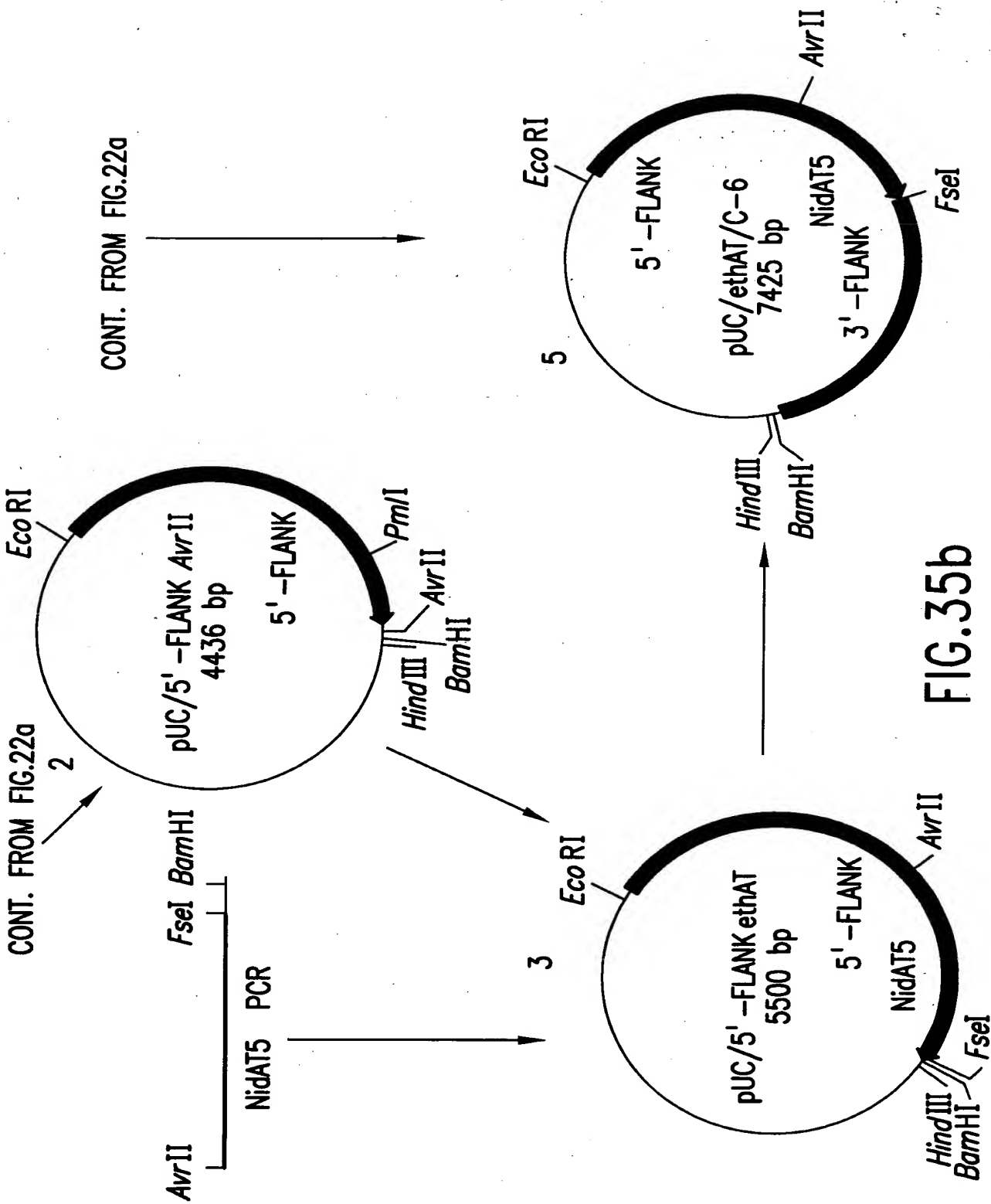


FIG. 35a



PROTEIN SEQUENCE	S	A	P	R	K	P
ORIGINAL SEQUENCE	TCCGCGCCGCGCAAGCCG					
				↓	↓	↓
ALTERED SEQUENCE	TCCGCGCCTAGGAAGCCG					
				└──────────┘		
				AvrII SITE		

PCR OLIGOS FOR 5'-FLANK *AvrII* SITE

N-TERMINAL OLIGO 5'-GAGAGAGGAACCAACGCGCACGTGATCGTCTGAAGAGGCACCAGC
(SEQ. ID. NO. 21)

┌──────────┐ 5'- FLANK SEQUENCE
 └──────────┘ *PmII* SITE

C-TERMINAL OLIGO 5'-GAGAGAGGATCCGACCTAGGCGCGGAGGTCACCGGCGCGACGGCG
(SEQ. ID. NO. 22)

┌──────────┘ 5'-FLANK SEQUENCE
 └──────────┘ *Bam*HI SITE *AvrII* SITE

PCR OLIGOS FOR *NidAT5* FRAGMENT

N-TERMINAL OLIGO 5'-GAGAGACCTAGGAAGCCGGTGTTCTGTTCCCCGGCCAGGGCT
(SEQ. ID. NO. 23)

┌──────────┐ BEGINNING OF *NidAT5*
 └──────────┘ *AvrII* SITE

C-TERMINAL OLIGO 5'-GAGAGAGGATCCGAGGCCGCGCCGTGCGCCCGGACCGAAGACCGCCTC
(SEQ. ID. NO. 24)

┌──────────┘ 3' END OF *NidAT5*
 └──────────┘ *Bam*HI SITE *FseI* SITE

FIG.36

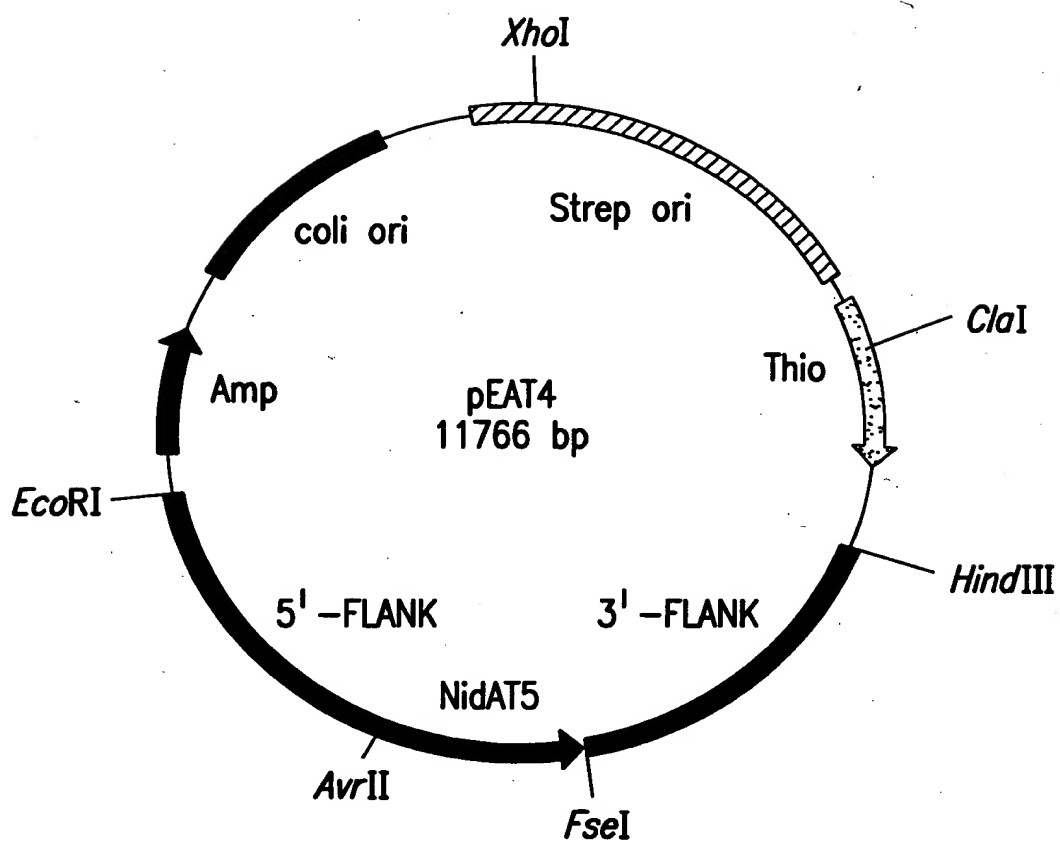


FIG.37

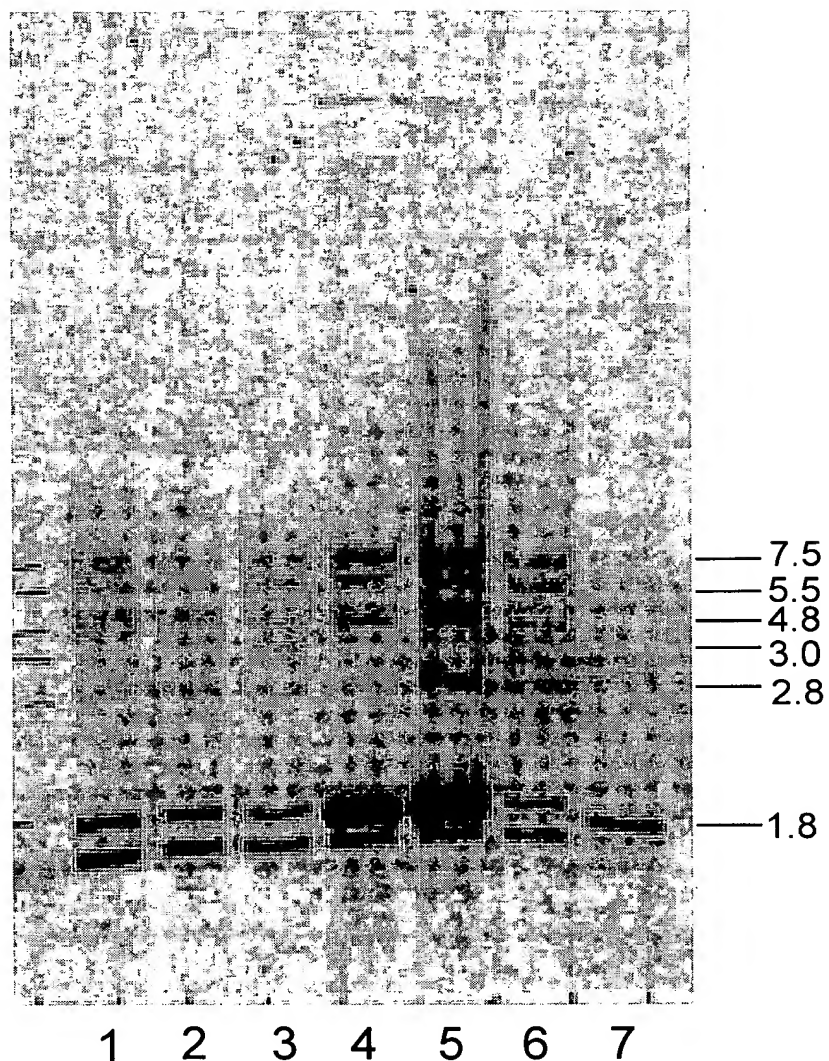


FIG. 38

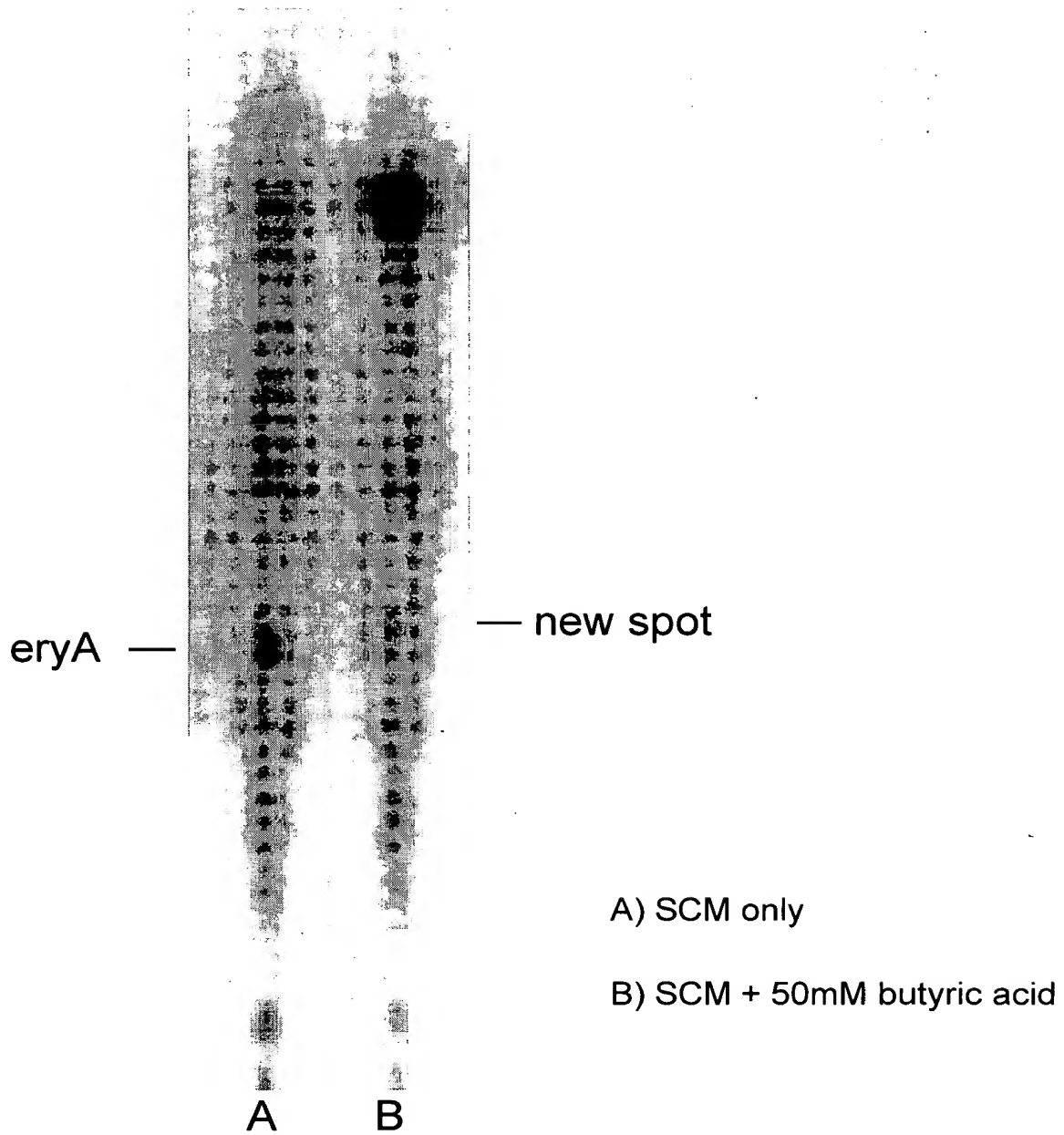


FIG. 39

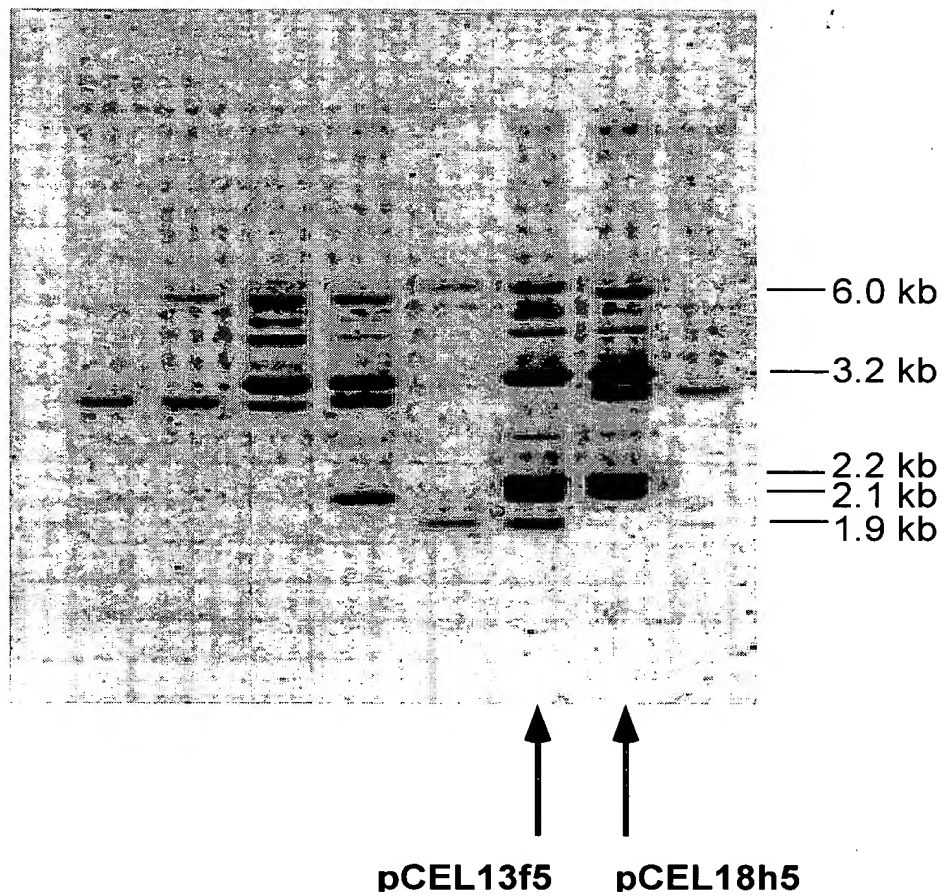


FIG. 40

CGCGCGCTGCCTTCGTCTTTCCCGGGCAGGGCGCCAGTGGGCCGACTGGGAGCGCGG 60
 R A P A F V F P G Q G A Q W A G L G A R 20
 CTCCTCGCGGACTCCCCGTCTTCCGCGCCAGGGCCGAGGCATGCGCGCGGGCGCTGGAG 120
 L L A D S P V F R A R A E A C A R A L E 40
 CCTCACCTCGACTGGTCCGTCTCGACGTGCTGGCCGGCGCCCCGGGCACCCCTCCCATC 180
 P H L D W S V L D V L A G A P G T P P I 60
 GACCGGGCCGACGTGGTGCAGCCGGTGCTGTTACACAGATGGTCTCGCTGGCCGCCCTC 240
 D R A D V V Q P V L F T T M V S L A A L 80
 TGGGAGGCCACGGGGTCCGGCCGGCCGGTCTGGGCCACTCCCAGGGCGAGGTGGCC 300
 W E A H G V R P A A V V G H S Q G E V A 100
 GCGCCCTGCGTGGCCGGTCCCCTGTGCTGGACGACGTGCCCTGGTGATCGCCGGACGC 360
 A A C V A G A L S L D D A A L V I A G R 120
 AGCAGGCTGTGGGGCGGCTGGCCGGGAACGGCGGGATGCTCGCGGTGATGGCTCCGGCC 420
 S R L W G R L A G N G G M L A V M A P A 140
 GAGCGATCCGTGAGCTGCTCGAACCATGCCGGCAGCGATTTCGGTGGCGCGGTCAAT 480
 E R I R E L L E P W R Q R I S V A A V N 160
 GGCCCCGCCTCGGTACCGTCTCCGGTGACCGCTCGCGCTGGAGGAGTTCGGCGCGCGG 540
 G P A S V T V S G D A L A L E E F G A R 180
 CTCTCCGCCGAGGGGTGCTGCGCTGGCCGCTGCCGGCGTCTGACTTCGCCGGCCACTCG 600
 L S A E G V L R W P L P G V D F A G H S 200
 CCGCAGGTGGAGGAGTTCC GC5CTGAGCTCCTGGACCTGCTCTCCGGCGTACGGCCGGC 660
 P Q V E E F R A E L L D L L S G V R P A 220
 CCTTCGCGGATACCTTTCTTCTCCACCGTGACGGCGGGTCTTGCGGCGGCGACCAGCTG 720
 P S R I P F P S T V T A G P C G G D Q L 240
 GACGGGGCGTACTGGTACCGCAACACGCGGAACCCGTGGAGTTCGACGCCACGGTCCGG 780
 D G A Y W Y R N T R E P V E F D A T V R 260
 GCGCTGCTGCGTGGGGCCATCACACGTTTCATCGAGTCCGTCCGCATCCGCTGCTCAAC 840
 A L L R A G H H T F I E V G P H P L L N 280
 GCCCGCATCGACGAGATCCGACGGACGAGGGGGTAGCGGCCACGGCCCTGCATACGCTC 900
 A A I D E I A A D E G V A A T A L H T L 300
 CAGCGGGCGCTGGCGGCCTTGACCGCGTGCGCAACGCGGTGGGCGCCGCTTTCGCGCAC 960
 Q R G A G G L D R V R N A V G A A F A H 320
 GGTGTCCGGTCCGACTGGAACGCCCTGTTTCGAGGGCACCGGTGCGCGCAGGGTGCCGCTT 1020
 G V R V D W N A L F E G T G A R R V P L 340
 CCCTCGTACGCCTTC 1035
 P S Y A F 345

FIG. 41

PCR OLIGOS:

N-TERMINAL OLIGO: 5' *Eco*RI Tag-^{*Avr*II}
 CCTAGGGTCGCCTTCGTCTTTCCCGGGCAGG-3'
 GCGC CCT

ENGINEERED <i>Avr</i> II AND <i>Va</i> I CODON	HOMOLOGOUS REGION
---	-------------------

C-TERMINAL OLIGO: 5' *Bgl*II Tag-^{*Nsi*I}
 ATGCATACGAGGGAAGCGGCACCCTGC-3'
 G G

ENGINEERED <i>Nsi</i> I	HOMOLOGOUS REGION
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PCR CLONING:

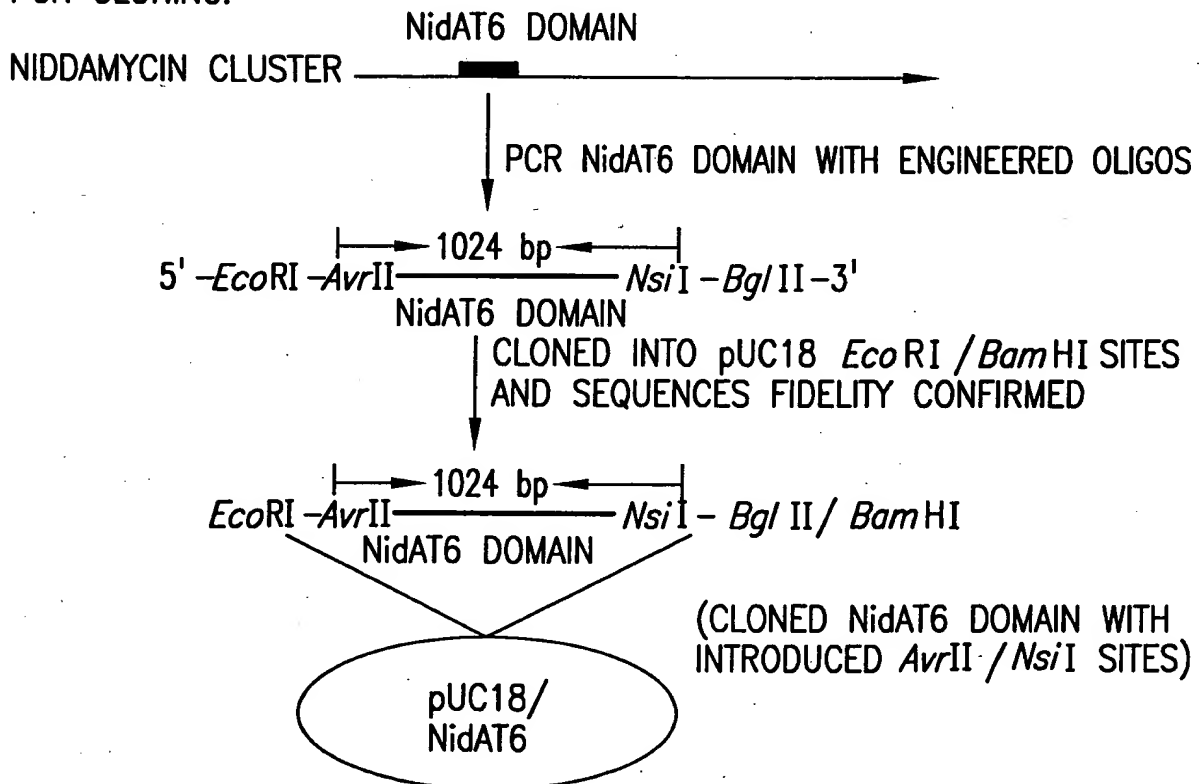


FIG.42

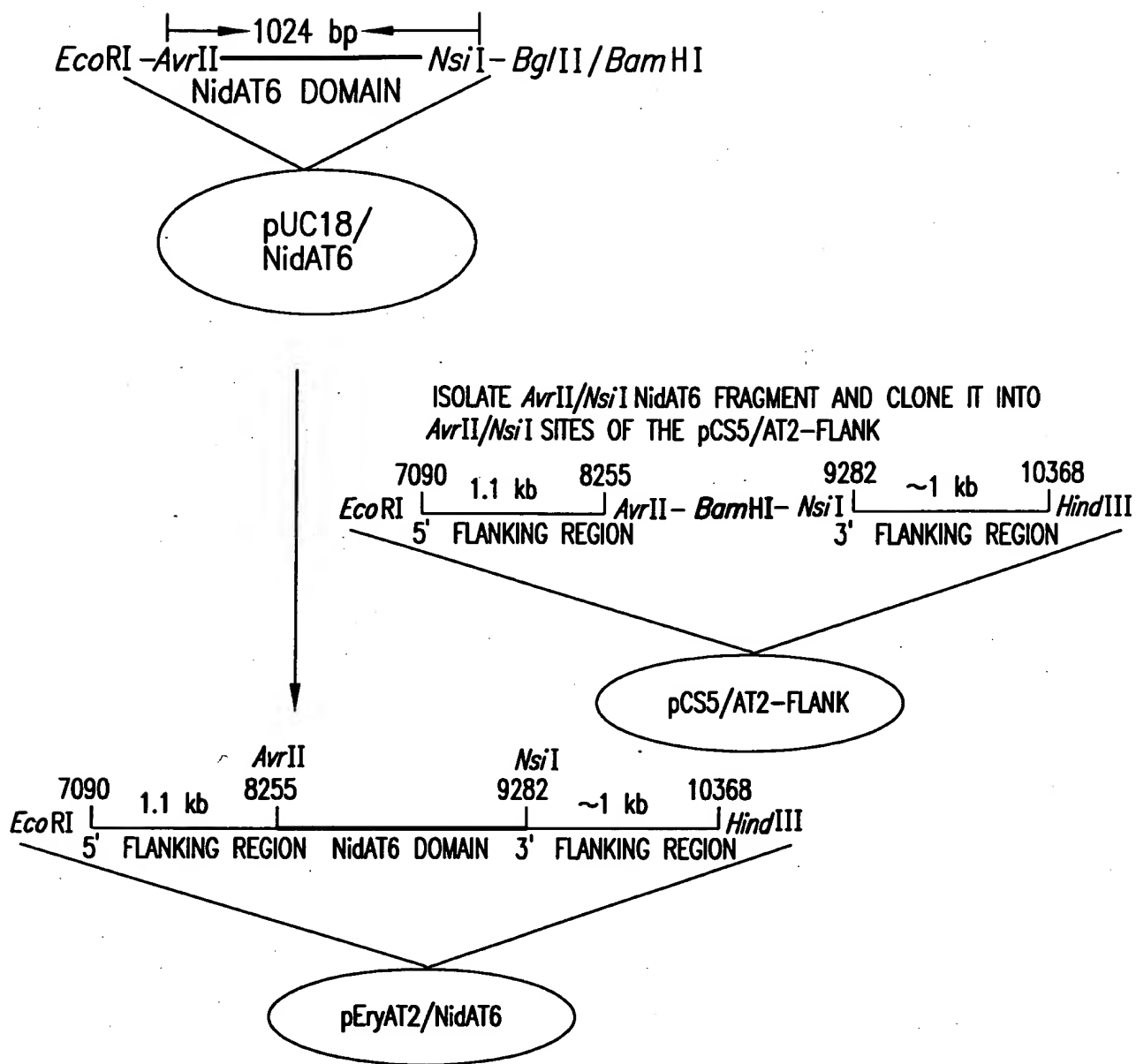


FIG.43